The role of lateral exchange in modulating the seaward flux of carbon, nitrogen and phosphorus.

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Queen Mary University of London
University of Essex
University of York
Imperial College London
University of Southern Denmark
Lancaster University

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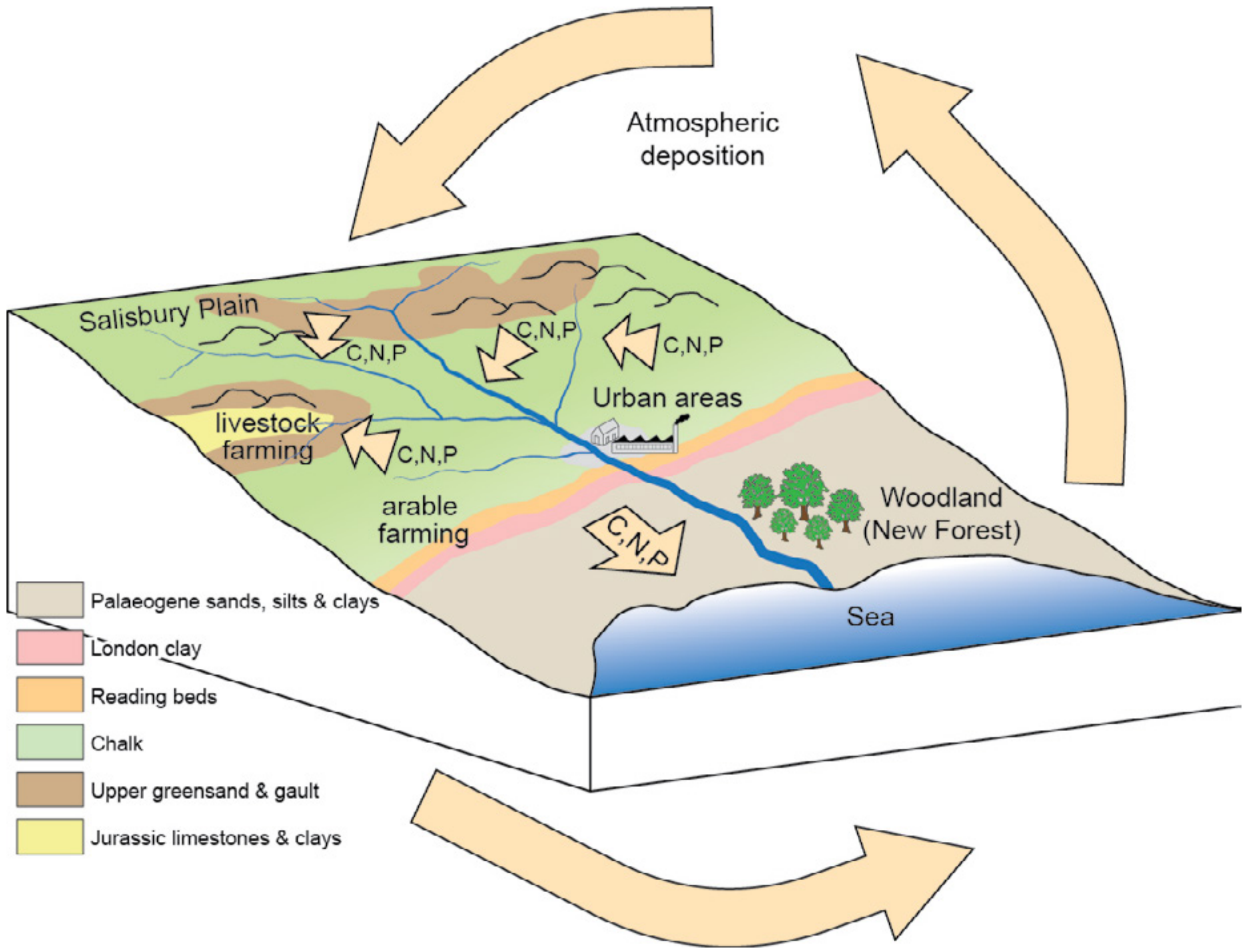
All living organisms that make up life on Earth are made from a profusion of elements in the periodic table. However, in addition to oxygen (O) and hydrogen (H), the constituents of water, the three most important are carbon (C), nitrogen (N) and phosphorus (P). These have become known as the macronutrients. These macronutrients are in constant circulation between living organisms (microbes, plants, animals, us) and the environment (atmosphere, land, rivers, oceans). Until human intervention (circa post industrial revolution and even more so since WWII) these 'cycles' were largely in balance: plants took up CO_2 and produced O_2 and, in order to do so, took up limited amounts of N and P from the environment (soils, rivers). On their death, this "sequestered" C,N,P was returned back to the Earth. Now human activity has put these key macro-nutrient cycles out of balance. Vast quantities of once fossilised carbon, taken out of the atmosphere before the age of the dinosaurs, are being burnt in our power stations and this has increased atmospheric CO_2 by about 30 % in recent times. More alarmingly, perhaps, is that man's industrial efforts have more than doubled the amount of N available to fertilize plants, and vast amounts of P are also released through fertilizer applications and via sewage. As the global population continues to grow these imbalances in macronutrient cycles are set to worsen.

Our project has studied this imbalance of macronutrients in the context of a lowland agricultural environment in the UK: the Hampshire Avon. We have made measurements to understand the different pathways by which macronutrients are travelling from grassland fields through the river system and out to the coast in different parts of the landscape – including on clay, sand and chalk geologies. We have also focused on the extent to which these macronutrients are being transformed in rivers along their route to the sea and, in particular, whether they are altered to form harmless chemical species or potent greenhouse gases. And we have measured the bacteria and environmental factors that control these transformations and alter the magnitude, rate and timing of the transport and transformation processes throughout the year.

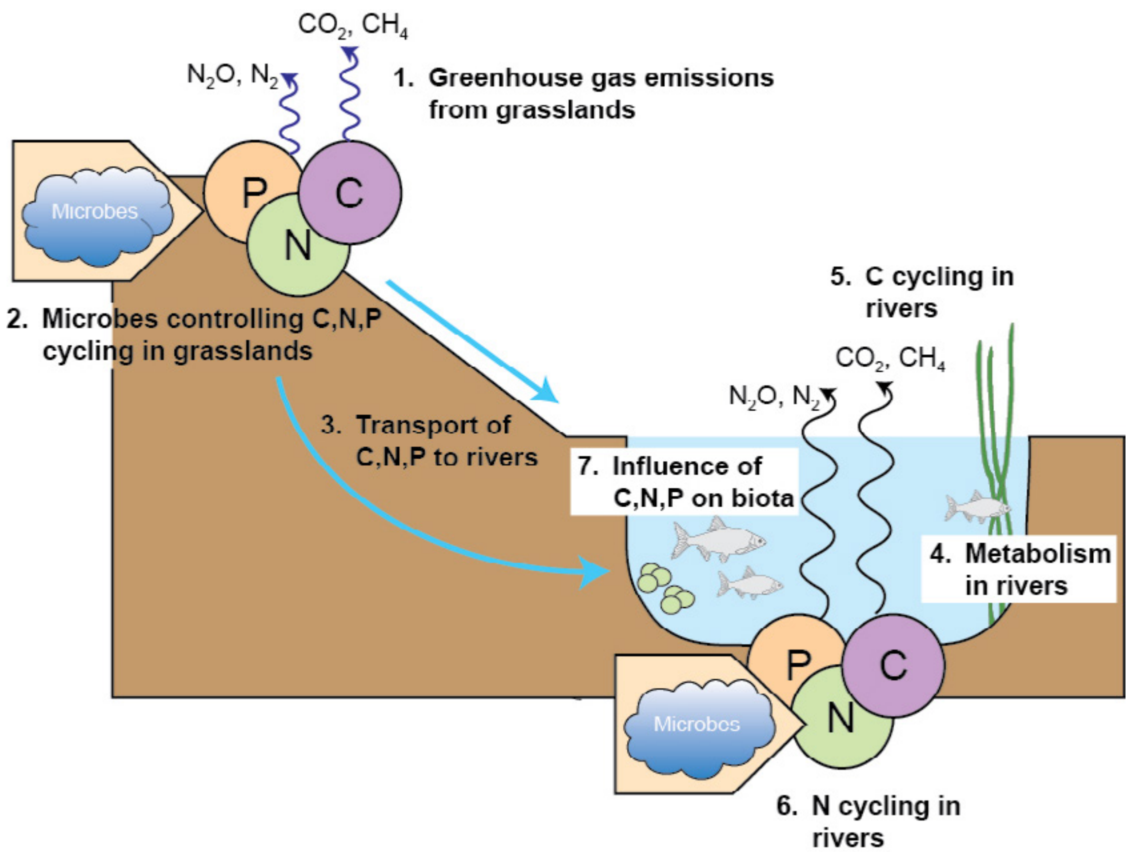


Macronutrient movement from catchment to coast.

In the UK our rivers provide a broad spectrum of local services, which spread from offering recreational areas, to supporting fisheries and providing landmarks. Chalk rivers, for example, are considered to have particular aesthetic and recreational value. At the global scale, rivers have been considered as passive pipes transporting carbon and other nutrients from the land downstream towards the coast. Recent studies, however, have suggested that streams can play a considerable role in, not only the transport, but also the transformations of carbon and nitrogen. For example by altering nitrate to harmless N_2 gas, or carbon from soils to carbon dioxide and/or methane gases. Therefore it is crucial to advance our understanding of the functioning and health of rivers such as the Hampshire Avon in order to assess the cycling of carbon, nitrogen and phosphorus at much larger scales.

This document outlines the various aspects of our three year research project and the findings that we consider to be most important contributions to scientific understanding. It is written by our team in a manner that we hope is accessible to those with little scientific knowledge of the subject, but with an interest in environmental science and management.

If you would like further information contact Kate Heppell at School of Geography, Queen Mary University of London (c.m.heppell@qmul.ac.uk) or Mark Trimmer at School of Biological and Chemical Sciences, Queen Mary University of London (m.trimmer@qmul.ac.uk).



A diagram to show the different aspects of our project as described below.

1. Greenhouse gas emissions from grasslands

How are greenhouse gas emissions affected by addition of fertilisers and by soil warming?

James Stockdale and Phil Ineson (University of York)

Our biggest challenge was to design, build and run a new system for measuring the movement of greenhouse gases from the soil to the atmosphere. Carbon dioxide, methane and nitrous oxide are all greenhouse gases that are contributing to climate change arising from human activity. Scientists predict that climate change will lead to increasing air and soil temperatures in the UK.

So we measured carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) at three sites with different geologies (chalk, clay, and greensand) to see how the soil and plants within each plot responded to warmer temperatures and to the addition of common fertilisers. One important feature was to be able to measure lots of experimental plots with a single automatic 'chamber' (think of an upturned perspex bucket!) which manoeuvred along suspended cables. This meant we could make measurements 24 hours a day, 7 days a week without the cost normally associated with lots of separate chambers or repeated trips to each site.

Our results show that the chalk and clay soils respond quite differently to fertiliser addition. Chalk soil produced the most N_2O when ammonium nitrate was added, but the clay soil continued to release N_2O for several days after its application. On its own, phosphate didn't have a big effect on N_2O but when combined with ammonium nitrate, the greensand soils also gave a large initial release of N_2O. Warming the plots by just 2-3 °C, during the dry summer of 2015, caused the grass to die back, photosynthesis (uptake of CO_2) to stop, and increased the overall release of CO_2. The chalk soils didn't suffer from this problem as much as the other sites, suggesting they were already better at coping with dry conditions, which might give grassland on chalk better chance of survival or continued healthy growth under future climate change scenarios for the UK.

Our novel gantry system for measuring greenhouse gas emissions from soils.

2. Microbes controlling C,N,P cycling in grasslands.

What are the ecological responses of microorganisms responsible for producing greenhouse gases?

Corinne Whitby, Dave Nedwell, Garwai Leung & Boyd McKew (Essex University)

The production of greenhouse gases in soils is caused by soil micro-organisms such as bacteria using carbon and nitrogen as an energy source. As part of the process, these micro-organisms transform these macronutrients from one chemical form to another. Our challenge was to determine how the bacteria in soils overlying clay, greensand and chalk varied in diversity, abundance and activity over a yearly cycle. It is important to measure these properties of the soil micro-organisms in order to fully understand how the production of greenhouse gases might vary in response to climate change. Diversity is a measure of the different types of microorganisms, abundance is the number of the microorganisms and the activity measures how active the microorganisms are in their environment. In order to measure these properties we collected soils from the same sites as described above, and extracted the genetic material from the bacteria present in the soil.

We found an abundance of bacteria in our soils. There are approximately 1-10 billion bacteria per gram of soil and the bacterial numbers were generally similar across geologies. In our soils the removal of nitrate by denitrification is controlled by soil temperature and water content – both environmental factors that are likely to vary in the future due to the effects of climate change. We also found the formation of the two greenhouse gases, nitrous oxide and methane, only occurred at significant levels in the clay soils. Methane can be removed from soils by oxidation processes which turn the methane into carbon dioxide, a less potent greenhouse gas. The rate of removal of methane by this process increased as temperature increased. Thus under climate change scenarios, clay soils are more likely to be better sinks for methane. However, we found that when fertilisers (such as nitrogen and phosphorus) were added to clay soils methane removal decreased. Therefore, the management of agricultural practices in clay soils is crucial, for future levels of greenhouse gases that will be released to the atmosphere.

3. Transport of C,N,P to rivers

How do macronutrients move from grassland riparian zones to the river?

Kate Heppell and Andy Binley (Queen Mary University of London and Lancaster University)

Rivers are strongly linked to the landscape that they flow through. River water was originally rain that has travelled from the surrounding land via soil and underlying rocks to the river channel. As the water moves through the soil it carries with it chemicals that it has encountered along its route, such as the macronutrients (nitrogen, phosphorus and carbon) which are the focus of our study.

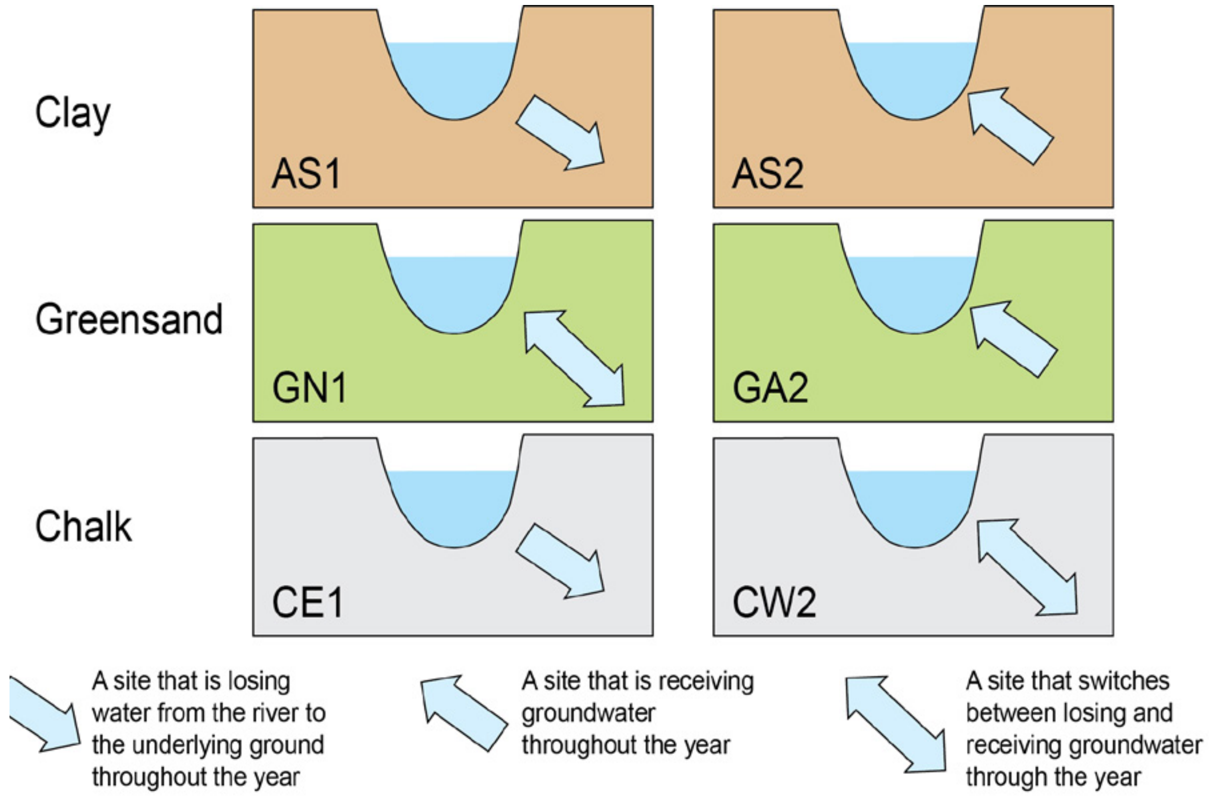
The Hampshire Avon is underlain by various different geologies, with chalk, greensand and clay being of particular interest to us because they have differing permeabilities (a measure of how easy it is for water to move through the soil or rock). Chalk has the greatest permeability and clay has the smallest. Thus water travels through landscapes made of these different geologies via different routes. In a landscape underlain by chalk much of the rain will travel through the soil to the underlying chalk aquifer and may take decades to reach the river channel. In a landscape dominated by clay the rainfall can make its way to the river rapidly by flowing over the soil surface or through man-made drainage channels that have been created to make farming possible on these low permeability, and often waterlogged, soils. We were interested in the consequences of these different pathways for the concentrations and quantity of macronutrients (carbon, nitrogen and phosphorus) reaching the river channel. We also wanted to understand how changes in the pathways by which water travels to the river channel during different seasons affects the transport of the nutrients.

To do this we installed probes at each of our sites to measure water quality along with automatic water samplers to take water samples at pre-programmed time intervals. We also measured water height in the river to calculate river discharge and we installed pipes (called piezometers) in the river to measure the direction that water flowed into and out of the river during different times of the year. These pipes also allowed us to measure water quality in the riverbed itself.

We found that the link between groundwater and the river varied across our six sites. At some sites our rivers received groundwater throughout the year, whilst others were continuously losing river water to the ground. Two of our sites switched between the river losing water and river gaining water depending on the time of year.

We found that the concentration of nitrate in our rivers increased as the permeability of the landscape increased. This may be a legacy issue, due to a history of intensive farming practices in the UK. It takes decades for rainwater to move from fields to the groundwater, and to the rivers in these catchments.

Piezometers to measure direction of water movement and water samplers next to the River Sem.



The losing and gaining character of our selected river monitoring sites.

4. Metabolism in rivers

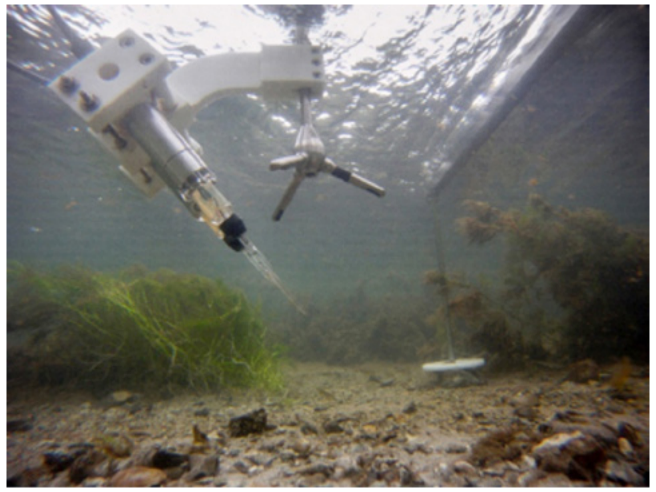
Measuring stream metabolism in the Hampshire Avon.

Lorenzo Rovelli and Ronnie Glud (Southern Denmark University)

One fundamental parameter of rivers to be assessed is the stream metabolism. This consists of the primary production by photosynthetic organisms, such as algae and aquatic plants, and the respiration (the action of breathing which produces CO_2) by fauna and microbial communities in the stream. Primary production is the creation of organic compounds, for example the growth of plant material in a river, primarily arising from photosynthesis (which uses light as a source of energy). These processes are highly dependent on the local environment and may differ substantially among rivers.

To assess conditions in the Avon river catchment we used the state-of-the-art methods to quantify and characterize stream metabolism at the streambed and in the water column. The activity in the streambed is quantified with a novel technique called eddy covariance, which enables activity measurements over a large area (tens of m^2) of the streambed, without altering the stream's natural flow temperature or light regimes. The technique represents a substantial improvement over traditionally more invasive approaches.

The investigations covered four seasons and revealed that each stream was characterized by highly varying rates of metabolism in the river water itself. This contrasted with the common conception that metabolism in river water is low because suspended algae and microbial densities are very low in comparison to the abundance of organisms (such as bacteria) in or on the streambed. We also observed a clear shift from net production of organic material during spring to periods of net degradation of organic material during autumn and winter. This would be related to processes such as the growth and die-back of aquatic plants in these rivers. Clear trends in stream metabolism were observed in the streams from clay river reaches, compared to river reaches underlain by greensand and chalk geology.



Eddy covariance system measuring river metabolism in the River Ebble.

5. Carbon cycling in rivers

Does carbon dioxide and methane production and rate of emission from rivers vary across geology?

Louise Olde, Mark Trimmer, Kate Heppell (Queen Mary University of London).

This component of our research has focussed on carbon cycling in the rivers of the Hampshire Avon. We were particularly interested in the rate at which carbon dioxide and methane (both greenhouse gases) were being produced at our different sites, and how the rates of movement of carbon dioxide and methane from the river to the atmosphere varied with different river conditions (e.g. seasonally or in response to rainfall).

We measured production of both carbon dioxide (CO_2) and methane (CH_4) in the riverbeds, in both light and dark conditions. At the same time we measured the flux of these gases from the river to the atmosphere (out-gassing). All riverbeds produced both CO_2 and CH_4, due to microbial activity in the sediment. However, the amount of CO_2 out-gassed to the atmosphere was much higher than could be explained by production in the riverbed; whilst for CH_4 the opposite was true, with much less being out-gassed than was produced in the riverbed.

This shows that the microbial production of these gases is not the most important factor in the flux to the atmosphere from the river. Instead, for CO_2 at least, atmospheric flux was more dependent on river conditions: during periods of high water flow after rain, much more CO_2 was out-gassed than under low flow conditions. This may be because CO_2-rich waters from the surrounding catchment get flushed into the rivers by the rain, and the CO_2 is then released to the atmosphere. At the same time, the production in the riverbeds is responsible for modulating the amount of flux between the day and night time. These findings should lead to a greater understanding of how these important greenhouse gases are transported between terrestrial and riverine ecosystems.

6. Nitrogen cycling in rivers

Do nitrogen cycling processes and microbes vary with geology?

Katrina Lansdown, Mark Trimmer, Kate Heppell (Queen Mary University of London), Corinne Whitby, Scott Warren, Graham Underwood & Boyd McKew (Essex University)

Not only did we consider carbon in rivers, but we also wanted to understand what happens to nitrogen once it enters a river. There is a global problem with excess nitrogen in surface waters, and the UK is no exception to this. It is important of us to understand the processes by which nitrogen, in its different forms, is transported to our rivers and the processes by which these different forms of nitrogen are transformed in our rivers. In particular, we want to understand whether excess nitrogen is simply converted from one form to another with no overall removal or are there processes which remove excess nitrogen permanently by making harmless N_2 gas which is emitted to the atmosphere? All of these macronutrient processes are driven by micro-organisms (such as bacteria and archaea) which use carbon and nitrogen species as their food or energy source.

The most exciting discovery in this research was that two process that remove nitrogen were occurring in rivers – denitrification and anaerobic ammonium oxidation (anammox). Denitrification is a much-studied pathway of nitrogen removal whereby micro-organisms convert nitrate to harmless N_2 gas. Anammox is also a microbial process but involves ammonium and nitrate reacting to form N_2 gas that previously was only thought to occur in estuaries, marine environments and wastewater treatment plants. As well as measuring nitrogen removal rates, we also studied the micro-organisms responsible for carrying out the anammox process. Many micro-organisms have preferences as to where they live or particular needs– for example in the case of bacteria that carry out anammox, it is widely thought that they prefer environments that are free from oxygen. In our rivers we found the presence of anammox bacteria with particularly high abundance of a particular gene called *hzo* (hydrazine oxidoreductase). This gene was associated with high rates of the anammox process. Our results were surprising to us because bacteria that carry out the anammox process are typically associated with environments that contain no oxygen unlike the oxygen-rich environments of chalk streams.

The contribution of anammox to total nitrate removal from rivers seems to vary across our different landscape settings; chalk, greensand and clay. We find anammox is most important in chalk rivers with a gravel substrate where land-river connectivity is low. Anammox is least important in clays were land-river connectivity is high (~30% and 5% of N_2 production, respectively). Finding that anammox is an active nitrogen removal process in rivers is important because compared to denitrification, anammox uses less energy to remove to nitrogen and does not produce N_2O (a potent greenhouse gas) as a by-product. We hope to keep researching anammox in river sediments in the future to better understand what environmental factors favour this nitrogen removal process.

We have also shown that microbial communities in clay and greensand river catchments have higher rates of respiration than photosynthesis, whereas chalk rivers have higher rates photosynthesis than respiration. Ultimately, this leads to different nutrient-processing functions, helping us understand that rivers may not all behave the same after nutrient addition.

7. Influence of C,N,P on biota

How do macronutrients influence invertebrates, algae and fish in the Hampshire Avon?

Octavian Pacioglu & Iwan Jones (Queen Mary University of London)

Another major task of this research project was to establish the extent to which C, N and P limit the growth and reproduction of invertebrates and fish in these streams. To achieve this, the biological components (invertebrates, algae, detritus and fish) at each site were sampled every two months for one full year. We measured the abundance and biomass (numbers and mass per m^2) of each species, and have used this information to determine their production (growth and reproduction of that species). We also looked at the C, N and P content of all food items, so that we can estimate the stocks of these elements in each system.

The next step was to establish what everything was eating. We did this in two ways:

a) by looking at the stomach contents of a sample of the fish (by pumping their stomachs) and invertebrates (by dissection), and

b) by using stable isotopes, naturally occurring tracers of carbon and nitrogen that are conserved between predators and prey.

Hence, we constructed a food web for each stream, where we knew how much of each food type was eaten by each species. As we knew the C, N and P content of the food, we could then work out how much C, N and P they were consuming, and relate this to their growth and reproduction.

Our results suggest:

1. The invertebrates and fish use a wider variety of food items in the chalk streams, where groundwater is an important source. In the clay streams, where food and nutrients enter the river from the land by surface and shallow subsurface flows, there is less variety of food and everything is competing for the same food.

2. Because there are a wider variety of things to eat, more species can co-exist in the chalk streams and hence the numbers and growth of fish is higher than in the clay rivers.

We have shown that the way the water flows bring nutrients from the landscape to the river influences the species and food webs of rivers.

Fish.

Light and temperature.

Invertebrates, algae, organic matter.

Samples of food web components were collected every two months.