Euro-limpacs

Project no. **GOCE-CT-2003-505540**

Project acronym: **Euro-limpacs**

Project full name: **Integrated Project to evaluate the Impacts of Global Change on European Freshwater Ecosystems**

Instrument type: **Integrated Project**

Priority name: **Sustainable Development**

Deliverable No. 52
Report on the use of climate controlled mesocosms for the incubation of intact cores in static chambers (Task 4.1)

Due date of deliverable: Month 18
Actual submission date: Month 24

Start date of project: **1 February 2002** Duration: **5 Years**

Organisation name of lead contractor for this deliverable: **ULIV**

Revision: Final

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level (tick appropriate box)		
PU	Public	PU
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

1. Introduction

Wetland ecosystems are currently under ecological stress from land-use change and pollution but may be subjected to additional pressures from climate change. Wetlands perform key ecological and socio-economic functions such as carbon sequestration and water quality amelioration. The IPCC predicts a 1.4 – 5.8 °C average increase in the global surface temperature over the period 1990 to 2100 (IPCC, 2001). Local changes in temperature and flooding may have dramatic impacts on wetland soil processes that may affect their function as regulators of river water quality and as contributors to global warming.

Nitrate (NO_3^-) is one of the key nutrients responsible for degradation of the quality of surface waters, principally through promotion of the process of eutrophication. Elevated concentrations in groundwater are a significant concern in many parts of the world. Nitrate has been implicated in a number of problems relating to human health including methaemaglobinaemia (Dudley, 1990), Non-Hodgkins lymphoma, miscarriages, stomach cancer (Britton *et al.*, 1990) and diabetes (McKinney, 1997). Nitrate itself is not directly toxic to humans but in the gut is converted to nitrite that bonds to haemoglobin. Also, elevated nitrate concentrations in surface water can cause qualitative changes in algal communities (i.e. blue-green algae) and oxygen depletion (Koo and O'Connell, xxxx) that may kill fish and invertebrates.

In the UK, agriculture is the main source of nitrate - approximately 75 % of nitrates released into most UK rivers and groundwaters (Defra, 2002; Powlson, 2000; House of Lords, 1989). There has been a long-term trend of rising nitrate concentrations in rivers in Central and Southeast England since the 1920s (Nitrate Coordination Group, 1986). Although nitrate levels in many surface water sources have stabilised in recent years (Davies and Sylvester-Bradley, 1995), they still remain high (Harris *et al.*, 2004; Beeson and Cook, 2004) and seasonal variations frequently exceed the EC limit which is 11.3 mg N l^{-1} (50 mg NO_3^- l^{-1}) in drinking water (EU Drinking Water Directive, 98/83/EC). As a worldwide average, pristine waters contain nitrate at approximately 0.1 mg N l^{-1} (Heathwaite *et al.*, 1996). UK aquifers have a natural background concentration less than 5 mg N l^{-1} (EA, 2005). Within Europe, Nitrate Vulnerable Zones (NVZ) have been designated in areas where these factors combine to produce a significant nitrate leaching risk, with farmers encouraged to implement management strategies aimed at reducing leaching (Davies, 2000).

River marginal wetlands convert nitrate leaching from agricultural land to nitrous oxide (N_2O) and dinitrogen (N_2) through the microbial process of denitrification. Facultative anaerobic, heterotrophic microorganisms carry out denitrification using nitrate as a terminal electron acceptor that results in the loss of nitrogen as gaseous nitrous oxide and dinitrogen:

$$C_6H_{12}O_6 + 4NO_3 \longrightarrow 6CO_2 + 6H_2O + 2N_2$$

The entire process actually involves a number of steps:

$$2NO_3^- \longrightarrow 2NO_2^- \longrightarrow 2NO \longrightarrow N_2O \longrightarrow N_2$$

where the intermediate might be nitric oxide (NO), though this is not certain. This is the primary type of dissimilatory nitrate reduction found in soil, and as such is of

concern because it cycles fixed nitrogen back into N_2O and N_2. Many bacterial genera possess membrane-bound enzymes for each step in the reduction of nitrate to nitrogen gas. Some take the reduction only to nitrite, and others may be unable to reduce nitrous oxide (Wild, 1993).

The removal of nitrate in marginal wetlands occurs as a result of both biological denitrification and assimilation by plants, although the latter process only occurs in summer (Burt and Haycock, 1993). The processes that operate within these ecosystems, due to their unique biogeochemical characteristics, means that they have great potential for removing nitrate from waters draining agricultural land, and preventing it entering the aquatic environment. Reduction of nitrogen in waters passing through wetlands has been reported as being regularly in excess of 80 % (Haycock and Burt, 1993).

The drawback to the positive effect of denitrification in wetlands on nitrate leaching from agricultural land is the emission of N_2O. Nitrous oxide is a potent greenhouse gas with a global warming potential 310 times that of carbon dioxide (CO_2) (IPCC, 1996) and a residence time of approximately 100 years in the atmosphere. Also, in the upper atmosphere, solar radiation can photolytically convert N_2O to nitric oxide (NO), which is a contributor to the depletion of the protective ozone layer. Thus there is potentially a dilemma in that changes in climate (i.e. temperature, flooding) that stimulate denitrification whilst enhancing river water quality may also stimulate N_2O emissions to levels that may have a positive feedback on global warming. Management strategies aimed at increasing the area of constructed marginal wetlands or re-establishing riparian zones to reduce river nitrate, may also promote global warming. Changes in temperature and flooding may also affect soil respiration of carbon dioxide (CO_2) and methane (CH_4) that may affect the carbon sequestration capacity of these wetlands.

An important question regarding denitrification in wetland soils is how climate change will affect the N_2O/N_2 ratio. Conditions that increase denitrification (i.e. nitrate reduction) whilst reducing the N_2O/N_2 ratio (i.e. N_2O reductase activity) would be favourable. During denitrification the N_2O/N_2 ratio decreases with increasing temperature (Nommik, 1956; Keeney *et al*.1979). This does not imply that a temperature increase results in a decrease in N_2O emissions from denitrification since an increase in total denitrification may outweigh any effect of a diminishing product ratio. Other factors also affect the N_2O/N_2 ratios. The rate of denitrification under more acid conditions is not only slower but the ratio of N_2O/N_2 is higher, either because of chemical reduction of nitrite to N_2O or inhibition of N_2O reductase. It has also been found that nitrate inhibits N_2O reductase, which results in a higher ratio of N_2O/N_2 in its presence (Wild, 1993). N_2O production is therefore promoted by nitrate availability because it is energetically favourable for denitrifyers to reduce nitrate instead of N_2O (Verhoeven *et al*., 2006). The ratio of N_2O to N_2 is also strongly influenced by soil water content, with a predominance of N_2O up to about 60 % water-filled pore space (WFPS), but a rapid increase in the relative production of N_2 so that it dominates above 80 % WFPS (Smith, 1997).

The work in this project aims to assess the effect of climate change on greenhouse gas emissions and denitrification. An increase in denitrification in river marginal wetlands under climate change scenarios would improve river water quality but may also result in positive feedback due to the emission of the greenhouse gas N_2O. However, whilst temperature and flooding may increase total denitrification, the emission of the greenhouse gas N_2O maybe further reduced to the end product N_2 that does not contribute to global warming. Thus determining the balance between total

denitrification of nitrate and N_2O/N_2 ratios under different climate change scenarios will provide relative information on conditions which promote beneficial (high denitrification; low N_2O/N_2 ratio) or detrimental (low denitrification; high N_2O/N_2 ratio) functioning.

Practical studies will involve the collection of soil cores from three types of floodplain wetland identified in the Tamar catchment and their incubation in static chambers under controlled conditions in the laboratory. Incubations will be carried out across a range of temperatures (0 – 25 ºC) and under both flooded and non-flooded conditions for greenhouse gas emissions and denitrification. The acetylene blockage technique will be used to measure denitrification rates and N_2O/N_2 ratios. Models will be developed (in the form of regression equations) for predicting denitrification rates and greenhouse gas emission rates from floodplain wetlands under future climatic and hydrological scenarios.

The hypotheses to be tested in this investigation are as follows:

Hypothesis 1 – Climate change in the Southwest of England will result in changes in temperature, rainfall and patterns of flooding.

Hypothesis 2 – Rates of denitrification, and hence N-transfer from agricultural land to the River Tamar will change as a result of climate change.

Hypothesis 3 – Fluxes of greenhouse gases from floodplain wetlands to the atmosphere will alter in the Tamar catchment as a result of climate change.

2. Methods

The field site is located at 'The Werrington Deer Park Estate'. This is a private country estate in the catchment of the River Otter, a sub-catchment of the River Tamar. Here the main river is of stream order 5 and the floodplain is up to 200m wide. This is one of the few locations in the whole of the Tamar catchment where three functional floodplain units occur within close proximity, in this case within 0.5km of each other. In the Tamar catchment, a total of eight different types of functional wetland units have been identified, comprising five 'slope' wetland units and three 'floodplain' wetland units (Hogan et al., 2000). As only floodplain wetland units are directly affected by overbank flooding from rivers (patterns of which will vary under changing patterns of rainfall), the work will focus on the effects of climate change (temperature and flooding) on these three floodplain wetland units. Additionally, this site happens to be located along one of the few stretches of river in the catchment that has two gauging stations relatively close to each other, in this case within approximately 3km. One of these is located upstream of the site and one downstream. Details of the location of the site and weather stations and stream gauging stations from which data is freely available are shown in Figure 1.

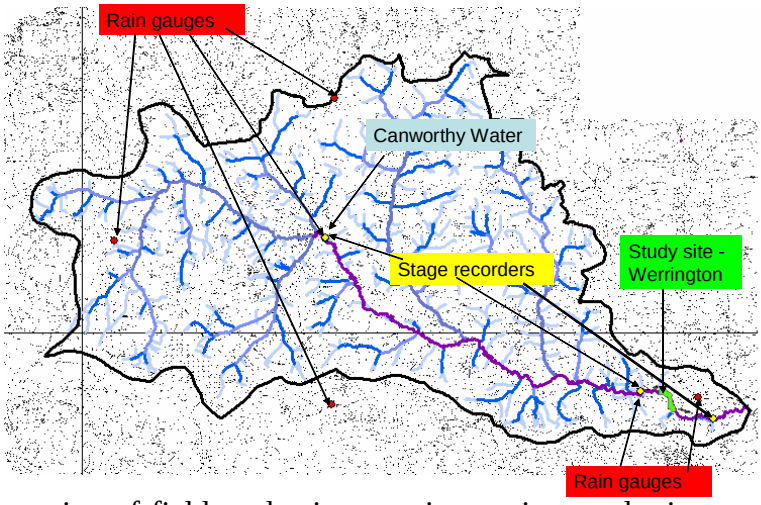

Figure 1. Location of field study site, gauging stations and rain gauges in the Ottery catchment, sub-catchment of the Tamar.

Practical investigations in the field and laboratory will be carried out over the period of one year, during which four assays representative of each season will be performed. Seasonal assays are deemed necessary to account for the temporal variations in factors that cannot easily be simulated in the laboratory (e.g. microbial dynamics, soil structure, etc.) throughout the year.

Replicate soil cores measuring 6.5cm diameter and 10 cm deep will be collected in perforated soil core liners from each of the floodplain wetland units being investigated. At the same time, soil pH will be measured. Five replicate soil cores from each location will be used to determine soil moisture content and bulk density by drying in an oven.

NH_4^+ and NO_3^- availability

In the laboratory replicate soil samples from each site will undergo KCl extraction to provide information on the nitrate and ammonium contents of the soils at the time of sampling. One set of extractions will be performed using standard KCl solution, and

another using nitrate amended solution in the presence of acetylene (denitrification during the shaking of a sample undergoing extraction can be relatively high and result in the incorrect determination of the quantity of extractable nitrate present in a soil). Water and extracts will be analysed for ammonium concentration using a Skalar segmented flow colorimetric analyser and for nitrate concentration using a Dionex liquid chromatographic analyser.

Greenhouse gas emissions and denitrifcation
Replicate soil cores from each wetland unit will be incubated in sealed kilner jar chambers at six different constant temperatures, both in the presence and absence of acetylene (to enable measurement of denitrification and greenhouse gas emissions respectively) and sequentially under non-flooded and flooded conditions over periods of 24 hours. The measurements of N_2O production with acetylene will allow determination of N_2 production allowing determination of the N_2O/N_2 ratio:

N_2O acetylene = Total denitrification (i.e. $N_2O + N_2$)
N_2O non-acetylene = Total N_2O produced
therefore,
Total N_2 produced = N_2O acetylene – N_2O non-acetylene

Headspace gas samples will be collected from each chamber at the start and end of each incubation period to determine flux rates. Headspace gas samples will be analysed for nitrous oxide, methane and carbon dioxide using a Perkin Elmer GC fitted with a automated sampler. Samples containing acetylene will be analysed for nitrous oxide only to determine rates of denitrification.

Data analysis
It is proposed that the data obtained from these experiments is used to derive seasonal regression equations for each wetland unit for rates of denitrification and gaseous emissions under both flooded and non-flooded conditions. This will be preceded by the performance of a range of descriptive statistical analyses (e.g. means, standard errors, comparative techniques, etc.) and procedures (e.g. normalisation of data, examination for autocorrelation). These data will be used to calculate seasonal emissions of greenhouse gases from and rates of denitrification in each wetland unit, based on both current patterns of flooding and temperature regimes and predicted future scenarios. The latter will use data provided by the Greek partners, EKBY, who are modelling the hydrology of the field site using historical hydrological and climatological data and predicted future climatological data. It is proposed that in each case annual fluxes/rates are calculated by deriving daily rates in each season and summing these to an annual rate.

Results will be extrapolated to a catchment scale using a GIS-based wetland inventory of the Tamar catchment, enabling comparison of the annual rates of denitrification and greenhouse gas emissions from floodplain wetlands under current and predicted future scenarios at a whole-catchment scale.

3. Results

Gas and water samples have been collected from cores incubated in climate controlled mesocosms for Autumn and Winter sampling periods. However, due to unexpected issues relating to the setup of analytical GC equipment at the University of Liverpool, delays have occurred on the running of gas and liquid samples collected. Field sampling is progressing as planned and the project is expected to finish within the deadline.

The project has been extended to incorporate laboratory based experiments addressing the impact of climate change on potential rates of denitrification (see Annex 1). These experiments will use the same treatment conditions as field experiments but examine potential denitrification using the denitrification enzyme assay (DEA) in parallel to measurements of N_2O flux under acetylene. Potential measurements estimate denitrification under conditions where carbon and nitrogen are non-limiting and thus provide maximal rates of denitrification. Also, experiments assessing the net flux of CO_2 and CH_4 (i.e. accounting for gross photosynthesis) are important for determining the emissions under climate change.

References

Beeson, S. and Cook, M.C. (2004) Nitrate in groundwater: a water company perspective. Quat. Jour. Eng. Geo. Hydro. 37, 261-270.

Britton, M., Fox, A.J., Goldblatt, P., Jones, D.R. and Rosato, M. (1990) The influence of socio-economic and environmental factors on geographic variations in mortality. In: Britton, M. (ed.). Mortality and Geography, OPCS Series DS No. 9 HMSO, London, 57-78.

Burt, T.P. and Haycock, N.E. (1993) Controlling losses of nitrate by changing land use. In: Burt, T.P., Heathwaite, A.L. and Trudgill, S.T. (eds.). Nitrate: Processes, Patterns and Management, John Wiley and Sons Ltd., Chicester, pp. 341-367.

Davies, D.B. (2000) The nitrate issue in England and Wales. Soil Use and Management 16, 142-144.

Davies, D.B. and Sylvester-Bradley, R. (1995) The contribution of fertiliser nitrogen to leachable nitrogen in the UK: a review. J. Sci. Food Agric. 68, 399-406.

Department for Environment, Food and Rural Affairs (2002) The Government's strategic review of diffuse water pollution from agriculture in England and Wales. Defra, London.

Dudley, N. (1990) Nitrates: The Threat to Food and Water, Merlin Press Ltd., London.

Environment Agency (2005) Attenuation of nitrate in the sub-surface environment. Science Report SC030155/SR2

Harris, R.C., Phillips, N. and Evers, S. (2004) Diffuse pollution from agricultural land: the need for integrated catchment management and radical rural land use change. In Hydrology: Science and Practice for the 21st Century. Volume II. British Hydrological Society International Conference, July 2004.

Haycock, N.E. and Burt, T.P. (1993) Role of floodplain sediments in reducing the nitrate concentration of subsurface run-off: a case study in the Cotswolds, UK. Hydrological Processes 7, 287-295.

Heathwaite, A.L., Johnes, P.J. and Peters, N.E. (1996) Trends in nutrients. Hydrological Processes 10, 263-293.

Hogan, D.V., Blackwell, M.S.A. and Maltby, E. (2000) Tamar 2000 SUPPORT Project: Wetlands Phase II Report. Unpublished report by the Wetland Ecosystems Research Group (WERG), Royal Holloway Institute for Environmental Research, Royal Holloway University of London. September 2000, pp. 39.

House of Lords (1989) Nitrate in water: with evidence. Select committee on the European communities. London: HMSO, pp. 288.

IPCC J.T. Houghton *et al*., (eds.). (1996) Climate Change 1995: The Science of Climate Change, Cambridge University Press.

Koo, B.F. and O'Connell, P.E. (XXXX) An integrated modelling and multicriteria analysis approach to managing nitrate diffuse pollution: 1. Framework and methodology. Sci. Tot. Env. Xxx-xxx.

McKinney, P. (1997) Nitrates and Childhood Diabetes. Diabetologia 40, 550.

Nitrate Coordination Group (1986) Nitrate in water. Department of environment, pollution paper. London: HMSO, pp. 104.

Powlson, D.S. (2000) Tackling nitrate from agriculture: foreward. Soil Use Management 16, 141.

Smith, K.A. (1997) The potential for feedback effects induced by global warming on emissions of nitrous oxide by soils. Global Change Biology 3, 327-338.

Annex 1

Factors controlling denitrification and greenhouse gas emissions in marginal wetlands

1. Denitrification and greenhouse gas emission preliminary laboratory experiments
2. Effect of temperature and flooding on potential denitrification and greenhouse gas emissions in riparian soils
3. Interactive effect of nitrate loading, temperature and flooding on greenhouse gas emissions and denitrification in riparian soils
4. Effect of temperature and flooding on net greenhouse gas emissions
5. Three-dimensional variation in denitrification in a river marginal wetland

Outlined in this section are experiments designed to determine the effect of climate change on denitrification, the end products (N_2O/N_2) of denitrification and emissions of greenhouse gases. Temperature and flooding are the major climatic changes facing natural ecosystems with carbon dioxide of less consequence to soil processes due to indirect interactions with plants (CO_2 interactions may require collaboration with other researchers in this workpackage). Nitrate loading is also an important factor since the input from agricultural runoff will ultimately determine N cycling in wetlands. Alteration to microbial N reactions by temperature and flooding may alter denitrification of different levels of nitrate loading.

1. Denitrification and greenhouse gas emission preliminary laboratory experiments

Soil core optimization

This experiment is required to determine the optimal (linear) incubation period for greenhouse gas emissions and denitrification. All subsequent experiments will utilise this incubation period.

Method
Collect 20 soil cores from River Tamar riparian zone.

Soil core incubations
Weight 20 cores. Place two reps in oven at 105 ºC for 2 days. Determine dry weight and soil moisture content. Place six replicates at either 0, 10 and 20ºC. For three replicates within each group add 10% acetylene to the headspace with the remainder serving as a control. For the acetylene cores, place under vacuum and purge headspace with N_2 for 10 mins. Collect gas samples at the following times: 0, 0.5, 1, 2, 4, 8 and 24 hrs. Total gas samples equals 126.

Nitrate and glucose incubations for DEA (potential)

Laboratory incubation experiments to be carried out to determine the optimal nitrate and carbon concentrations required for further analyses of potential denitrification (DEA).

Method
Collect 30 soil cores (upper 10 cm) from River Tamar riparian zone. Measure nitrate and ammonium using KCL extracts for 3 replicates. Perform all incubation at 10ºC.

Soil moisture (36 replicates)
Weight 2 x 25 g soil from each core into 250 ml headspace flasks so as to pair control and acetylene replicates. Add either 0, 10, 20, 30, 40 or 50 ml riverwater to six replicates. Homogenise into a slurry. For three replicates within each group add 10% acetylene to the headspace with the remainder serving as a control. Collect gas samples at 0, 0.5, 1, 2, 4, 8 and 24 hrs. Gas samples equals 252.

KNO_3 incubation (36 replicates)
Weight 2 x 25 g soil from each core into 250 ml headspace flasks and add optimal ml of riverwater. Homogenise into a slurry. To each of six replicates add either 0, 10, 50, 100, 250 or 500 µg N g^{-1} soil as KNO_3. For three replicates within each group add 10% acetylene to the headspace with the remainder serving as a control. Collect gas samples at 0, 0.5, 1, 2, 4, 8 and 24 hrs. Gas samples equals 252.

Glucose incubation (36 replicates)
Weight 2 x 25 g soil from each core into 250 ml headspace flasks and add optimal ml of riverwater. Homogenise into a slurry. To each of six replicates add either 0, 10, 50, 100, 250 or 500 µg C g^{-1} soil as *D*-glucose or sucrose. For three replicates within each group add 10% acetylene to the headspace with the remainder serving as a control. Collect gas samples at 0, 0.5, 1, 2, 4, 8 and 24 hrs. Gas samples equals 252.

Chloroampheniol
Add varying concentrations of chloroamphenicol to soil slurries (0, 50, 100, 200, 300, 600 µg g) and measure greenhouse gases and N_2O under acetylene as above.

N mineralization
Perform KCL extracts for nitrate and ammonium on 3 reps from each treatment.

Analysis
Plots of N and C against greenhouse gas emissions and denitrification will allow determination of optimal N and C concentrations.
Pair replicates according to greenhouse gas emissions.

Total time = 2 weeks

2. Effect of temperature and flooding on denitrification and greenhouse gas emissions

This experiment will determine the effect of temperature (0, 5, 10, 15, 20 and 25 ºC) and flooding (flooded and non-flooded) on greenhouse gas emissions (CO_2, CH_4 and N_2O flux) and denitrification (actual [acetylene] and potential [acetylene, NO_3^-, chloroamphenicol and glucose]).

An important question regarding impacts of nitrogen loading and climate change on denitrification is what conditions will favour N_2 production (i.e. optimal conditions) or N_2O production (i.e. incomplete denitrification due to suboptimal conditions). By examining the difference between N_2O flux and N_2O flux in the presence of acetylene it is possible to determine the amount of N_2 being produced:

N_2O acetylene = total denitrification (i.e. $N_2O + N_2$)
N_2O non-acetylene = total N_2O produced
Therefore N_2O acetylene – N_2O non-acetylene = total N_2 produced

These results are actually measurements of nitrate reductase activity (NO_3^- - N_2O) and nitrous oxide reductase activity (N_2O – N_2). Thus factors that increase complete denitrification to N_2 are preferential to incomplete denitrification to N_2O.

High N_2O/N_2 (>1) = detrimental
Low N_2O/N_2 (<1) = beneficial

Method
Collect 130 cores from River Tamar riparian zone.

Weight all cores (wet weight). Place 5 cores in oven at 105 ºC for determination of dry weights and soil water content. Incubate 10 cores at 0, 5, 10, 15, 20 and 25 ºC for 1 week. For 5 cores in each treatment maintain soil moisture twice daily by loss of weight (rainwater). For the remaining 5 cores flood the core completely with riverwater and flush with N_2.

Place 30 g of soil in ten 250 ml flasks at 0, 5, 10, 15, 20 and 25 ºC for 1 week. For 5 reps in each treatment add optimal N and C solution and distilled water to controls.

	Non-flooded		Flooded		
	Greenhouse	DEA	Greenhouse	DEA	
	+ acetylene		+ acetylene		
Temperature	CORES	SLURRIES	CORES	SLURRIES	
0	5	5	5	5	20
5	5	5	5	5	20
10	5	5	5	5	20
15	5	5	5	5	20
20	5	5	5	5	20
25	5	5	5	5	20
	30	30	30	30	120

For the cores determine greenhouse gas emissions over the incubation period determined in Exp. 1. Then add acetylene to all cores including slurries and determine emissions again. Total gas samples equals 360.

Analysis
Perform linear regression analysis of greenhouse gas emissions, actual denitrification and DEA against temperature under both non-flooded and flooded conditions (linear or exponential??). Derive Q_{10} values for each flux, and equations for modelling based on soil weight and area. Calculate the total global warming potential by adding each flux x GWP. Assess the global warming potential and N_2O/N_2 ratio under different temperature and flooding scenarios. Use the information to decide on two scenarios for use in Exp. 3 – impact of nitrogen loading. Apply regional climate models to the equations to determine impacts of climate change on greenhouse emissions and denitrification. Compare data to field data.

3. Interactive effect of nitrate loading, temperature and flooding on greenhouse gas emissions and denitrification in riparian soils

Using the data from Exp. 2 the effect of nitrate loading under two scenarios will be assessed. Cores will be incubated under different NO_3^- loadings for 6 months with periodic measurements of greenhouse gas emissions and denitrification taken every 2 weeks.

4. Effect of temperature and flooding on net greenhouse gas emissions

As part of the first project at the River Tamar, it may be necessary to determine net greenhouse gas emissions and soil respiration in the field. Greenhouse gas emissions in the first project were measures of soil respiration and thus did not take into account gross photosynthetic activity. Thus the effects of temperature and flooding on carbon dioxide emissions will be overestimated.

In situ net greenhouse gas emissions can be determined simply in the field using PVC chambers with acrylic lids and septa. Soil respiration rates can be determined by using black out cloth to inhibit photosynthesis. It is proposed that net ecosystem exchange (NEE) and dark (soil) fluxes of CO_2, CH_4 and N_2O are determined at each wetland type for each season (3 wetlands, 5 replicates, 2 gas samples per replicate = 30 gas samples). Then laboratory mesocosm experiments will determine the effect of temperature and flooding on NEE, gross photosynthesis and soil respiration. Regression equations could then be used to scale to field data and correct for plant processes.

5. Three-dimensional variation in denitrification in a river marginal wetland

Martin *et al*. (1999) point out that most research into denitrification in riparian zones focuses on only two dimensions: (1) in transects parallel to the stream and (2) linearly from the pollution source towards the stream. The two dimensional perspective neglects to consider the third, vertical, dimension of the riparian ecotone. Large masses of agricultural NO_3^- can be transported to depths of 16m below cultivated fields via ground water percolation. Thus, it is clear that subsurface riparian soils must be considered when examining the overall effectiveness of the riparian zone in terms of NO_3^- removal.

Whilst it has been shown that denitrification is viable at significant depths (see Martin *et al*., 1999), research is required to fully appreciate the capacity for river marginal wetlands to reduce NO_3^- pollution. Models based on the seasonal variation of denitrification in surface soils would not account for rates at depth that maybe buffered from temperature or under waterlogged conditions for most of the year.

Method

This experiment will be performed twice: in the summer 2006 and winter 2007. For each of the three riparian zones on the River Tamar, soil cores will be collected to a depth yet to be determined along a transect between the terrestrial ecosystem and the stream. Soil samples will be analysed for greenhouse gas emissions, denitrification and available NH_4^+ and NO_3^-.

Martin, T.L., Kaushik, N.K., Trevors, J.T. and Whiteley, H.R. (1999) Review: Denitrification in temperate climate riparian zone. Water, Air, Soil Pollution 111, 171-186.