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Denitrification within riverine systems of North-East England

A thesis submitted by Sarah Pattinson for the degree of Doctor of Philosophy in the University of Durham, England, May 1999

ABSTRACT

This study was undertaken to investigate denitrification and nitrous oxide production in sediments and the key environmental factors influencing these within selected river systems of the LOIS (Land-Ocean Interaction Study) area in North-East England and southern Scotland.

Seasonal and spatial trends were evident in both environmental and denitrification data measured monthly for 1.5 years along the Swale-Ouse system from source to tidal limits. Denitrification, measured in sediment cores using acetylene inhibition and expressed by unit area of sediment, increased with distance from source down to freshwater tidal limits. Results from a supplementary survey of the freshwater tidal reaches of the Yorkshire Ouse showed a decrease from the tidal limits. Denitrification activity showed a spring (March to May) peak, particularly in the lowland sites. The highest rate (883±134 μmol N m^{-2} h^{-1}) was measured on the River Wiske, a highly eutrophic lowland tributary to the Swale. A high degree of colinearity was evident between environmental variables, although a significant relationship between denitrification, nitrate and temperature was found through multiple regression.

For comparison, measurements were made in the less populated Tweed river system. The seasonal and spatial trends evident in both the environmental and denitrification data from the River Tweed, under a more limited sampling programme, were generally consistent with those observed in the Swale-Ouse system.

An intensive field investigation of 50 river sites showed that both potential denitrification rate and N_2O production in sediment slurries were positively correlated with nitrate water concentration, sediment water content and percentage of fine (<100 μm) sediment particles.

An experimental study investigating the kinetic parameters for denitrification, found that sediment cores taken along the Swale-Ouse exhibited a saturation type curve with added nitrate. Apparent affinity and estimates of apparent maximum velocity for mixed populations of denitrifying bacteria showed an increase on moving downstream and were highest on the Wiske.

Denitrification within riverine systems of North-East England

by

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A thesis submitted for the degree of Doctor of Philosophy in the University of Durham, England

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May 1999

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This thesis is entirely my own work and has not been previously submitted for any other degree.

Sarah Pattinson
May 1999

LIST OF ABBREVIATIONS

°C	degrees Celsius
g	gram
mg	milligram
µg	microgram
L	litre
mL	millilitre
µL	microlitre
km	kilometre
m	metre
cm	centimetre
mm	millimetre
µm	micrometre
M	molar
mM	millimolar
µM	micromolar
mmol	millimole
µmol	micromole
nmol	nanomole
meq L^{-1}	milliequivilents per litre
µS cm^{-1}	microsiemens per centimetre
i.d.	internal diameter
d.wt.	dry weight
v/v	volume by volume
ppbv	parts per billion by volume
y	year
d	day
h	hour
min	minute
s	second
SD	standard deviation
SE	standard error
n	number of replicates
p	probability
df	degrees of freedom
R^2	coefficient of determination
NS	not significant
K_m	Michaelis-Menten constant
K_s	apparent half saturation concentration

V_{max}	apparent maximum velocity
Eh	redox potential
DNRA	dissimilatory nitrate reduction to ammonium
ECD	Electron Capture Detector
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
TFP	Total filtrable phosphorus
FRP	Filtrable reactive phosphorus
FOP	Filtrable organic phosphorus
LOIS	Land-Ocean Interaction Study
RACS	River-Atmosphere-Coast Study
NORMS	North Sea Modelling Study
SES	Shelf Edge Study
LOEPS	Land-Ocean Evolution Perspective Study
IFE	Institute of Freshwater Ecology
IH	Institute of Hydrology
DANI	Department of Agriculture, Northern Ireland
NERC	Natural Environmental Research Council

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CHAPTER 1
INTRODUCTION

1.1 General comments

Inputs of nitrate to surface waters have increased steadily during recent decades (Meybeck *et al.*, 1989) as a result of a number of factors, including increased fertiliser application. Rivers may, therefore, be a major source of eutrophication in estuarine and coastal ecosystems. However, although there is no lack of information on the origin, cause and magnitude of nitrogen loading, there is a gap in current knowledge with respect to the effects and retention of increased nitrogen loading in aquatic systems.

As recipients of nitrogen, rivers can subsequently act as regulators of the flux of nitrogenous compounds from N-fertilised terrestrial ecosystems to estuarine and coastal marine environments. Denitrification is one potential pathway for removal of nitrate, and rates are sometimes high enough to be taken into account when preparing an overall nitrogen budget for a river (Kaushik & Robinson, 1976; Hill, 1981; Cooper, 1990). However, despite this importance Seitzinger (1988) commented that few studies on denitrification in streams and rivers had been made. Although a number of studies have been made since then, denitrification data for rivers appear to be still largely lacking when compared to the estuarine and marine environments.

This study was undertaken to investigate denitrification and nitrous oxide production in sediments and the key environmental factors influencing these within selected river systems of the LOIS (Land-Ocean Interaction Study) study area in North-East England and southern Scotland (see Appendix 1). This would ultimately contribute to the assessment of the significance of denitrification in these river systems.

1.11 Significance of nitrogen

Nitrogen and phosphorus are the two nutrients most likely to limit primary production in freshwaters and perform a major role in eutrophication of both freshwater and marine ecosystems (Wetzel, 1983). In estuaries and coastal areas, an increase in nitrogen loading will result in increased primary production that can subsequently result in oxygen deficits and even fish kills (Kessler & Jansson, 1994). For example, all these phenomena occur regularly along the coasts of Southern Sweden and Denmark as a consequence of an unnaturally high nitrogen flux (Jansson *et al.*, 1994). As recipients of run-off from terrestrial ecosystems, rivers may be a major source of nutrients to the estuarine and coastal ecosystems, resulting in such deleterious environmental effects.

There is currently a steady increase in nitrogen loading in the freshwater environment (Torre *et al.*, 1992), resulting in high land-to-sea fluxes. This is the result of a number of factors, such as increased nitrogen fertiliser application, changes in land use, increased recycling of domestic wastewater and atmospheric deposition (Wilkinson & Greene, 1982; Heathwaite *et al.,* 1994). For example, on a global scale, Meybeck (1982) suggested that the average concentration of nitrogen for world rivers in a 'pristine state' is 0.015 mg L^{-1} NH_4-N, 0.001 mg L^{-1} NO_2-N and 0.1 mg L^{-1} NO_3-N. A report published jointly by the United Nations Environment Programme (UNEP) and the World Health Organisation (WHO) in 1988 found in some regions of the world, particularly Europe, less than 10 % of rivers could be classified as pristine on this basis. This not only has implications for human health, for example methaemoglobinaemia and stomach cancer, but also for the environment, resulting in EC Directives such as the Drinking Water Directive (COM 80/788), the Nitrates Directive (COM 91/676), and the Urban Wastewater Treatment Directive (91/27/EEC). These Directives are concerned with the control of nitrate in receiving waters and encourage the adoption of practices compatible with environmental protection. Thus, as stated above, there is abundant information relating to the origin, cause and magnitude of nitrogen loading, but a gap in current knowledge with respect to the effects and retention of increased nitrogen loading in aquatic systems. Without data to illustrate the fate of excessive nitrogen in limnetic systems, difficulties in introducing nitrogen transport reduction measures would arise.

It is evident from a number of reports that mechanisms for the reduction of nitrogen loading should be put in place; for example, Postma (1985) reported that the load of combined nitrogen transported by rivers to the North Sea had increased seven-fold in the last 50 years. It has also been estimated that, on a global basis, rivers transport 24 Tg y^{-1} N to the ocean, 80% of which is estimated to be the result of human activity (Wollast, 1983). This riverine flux of nitrogen to the oceans is 60 % higher than the total annual contribution of benthic nitrogen fixation (Capone, 1983). Thus, any mechanisms that reduce riverine nitrogen load will reduce nitrogen inputs to the oceans, therefore alleviating eutrophication problems. It is hence becoming increasingly important to understand the processes that lead to the removal of nitrate from aquatic systems.

There has been much study into the processes involved in the nitrogen cycle and the relationship between nitrogen and phosphorus (*e.g.* Schindler *et al.*, 1973; Lund, 1981; Schindler, 1988). Additionally, there has also been substantial research into mechanisms to reduce phosphorus concentrations in streams and waterways, for example through the introduction of tertiary treatment in many sewage-treatment plants. However, despite its potential deleterious environmental effects, there has been little research with regard to reduction of nitrogen.

1.12 Aquatic nitrogen cycle

Nitrogen (N) in freshwater is found in several forms, the dynamic interrelationships of which can be described as the aquatic nitrogen cycle. The dominant forms of combined N in waters (excluding molecular N_2) are dissolved inorganic N (NH_4^+, NO_2^-, NO_3^+), dissolved organic N, and particulate N, which is usually organic but can contain inorganic N. The transport of nitrogen through these different forms, processes and reservoirs during the nitrogen cycle ultimately regulates the cycle of organic matter, as nitrogen is an essential component of all living organisms. There are five processes within the nitrogen cycle: fixation, assimilation, ammonification, nitrification and nitrate reduction (Fig. 1.1). Nitrate reduction includes both dissimilatory nitrate reduction to ammonium (DNRA) and denitrification.

1.2 Denitrification

Denitrification performs a significant function within the nitrogen cycle, as it is the only biologically-mediated process that terminates in the production of gaseous forms of nitrogen. It effectively works in opposition to the process of nitrogen fixation, where N_2 is microbially converted to organic nitrogen. By removing nitrogen completely from the aquatic system, denitrification reduces the flux of nitrogen from land to coastal waters. This makes denitrification an important natural process in reducing the potential problems generated by eutrophication as discussed above (see Section 1.11).

Denitrification is a microbially-mediated process that results in the reduction of one or both of the ionic nitrogen oxides (nitrate or nitrite) to two of the gaseous oxides (nitric oxide and nitrous oxide). These gaseous oxides may in turn be further reduced to

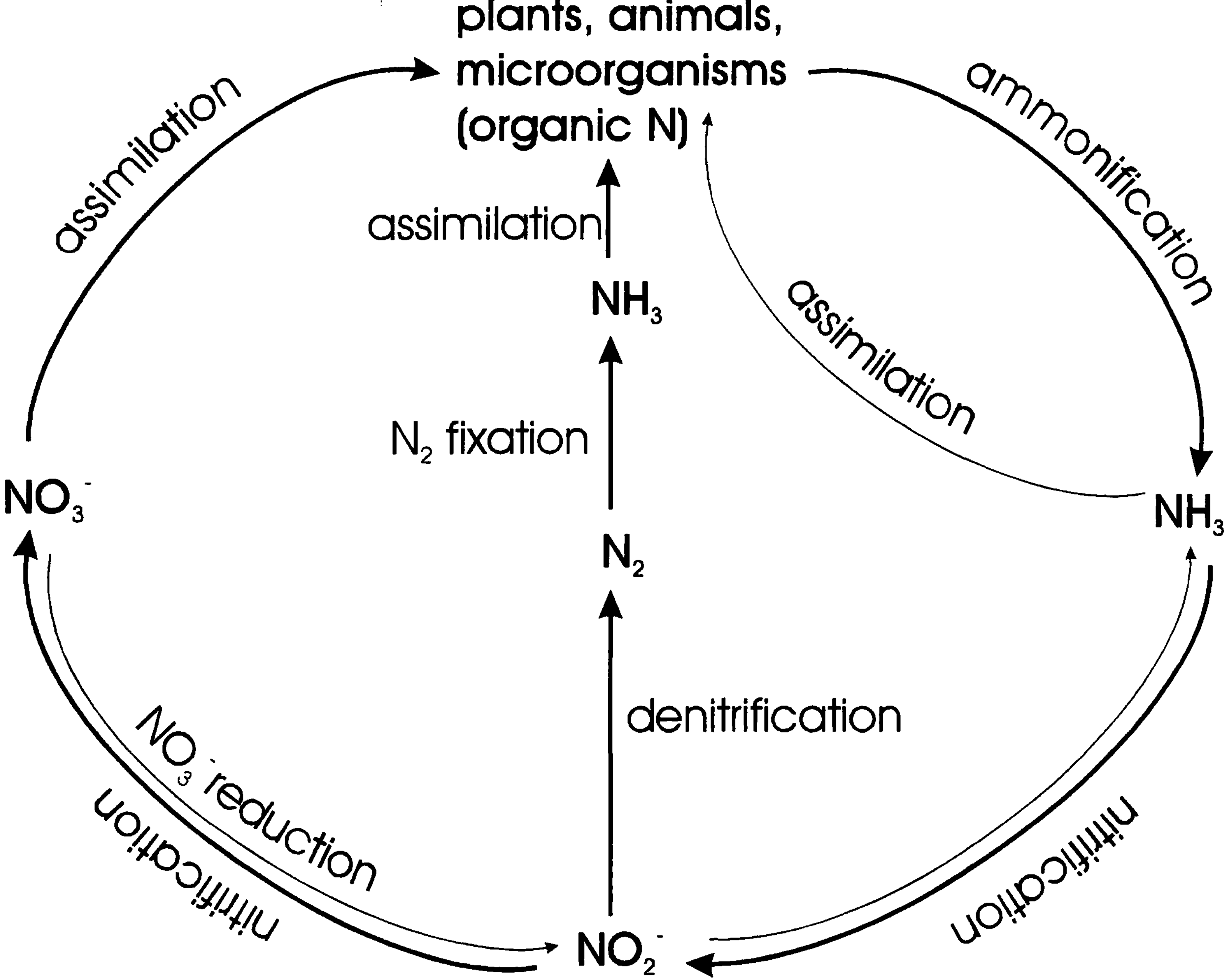


Figure 1.1 A generalised N cycle, showing the major processes involved

N_2. This pathway was first suggested by Payne (1973) and is summarised below in Equation (1).

$$NO_3^- \underset{\substack{\text{nitrate}\\\text{reductase}}}{\Rightarrow} NO_2^- \underset{\substack{\text{nitrite}\\\text{reductase}}}{\Rightarrow} NO\uparrow \underset{\substack{\text{nitric}\\\text{oxide}\\\text{reductase}}}{\Rightarrow} N_2O\uparrow \underset{\substack{\text{nitrous}\\\text{oxide}\\\text{reductase}}}{\Rightarrow} N_2\uparrow \qquad (1)$$

Denitrification is carried out by facultatively anaerobic bacteria that are both biochemically and taxonomically very diverse. Denitrifiers are heterotrophs that use the nitrogen oxides as terminal electron acceptors to generate energy (ATP) through anaerobic respiration under low oxygen conditions, using specific enzymes as catalysts (Knowles, 1982; Rysgaard *et al.*, 1994). Synthesis of these denitrifying enzymes is controlled by ambient oxygen levels, and the oxidised nitrogen form available for reduction (Körner & Zumft, 1989). Most of these bacteria possess all of the reductases necessary to reduce NO_3^- to N_2, although some lack nitrate reductase and are therefore classified as NO_2^- dependent. Others lack nitrous oxide reductase and can only produce nitrous oxide (N_2O) as a final product. The most commonly isolated denitrifying bacteria from both plants and soils belong to the genus *Pseudomonas* (Heitzer & Ottow, 1976; Gamble *et al.*, 1977) and it is assumed that these are the most active denitrifiers in natural environments.

Due to the fact that reduced oxygen conditions, even anoxic conditions, are required for the reduction of NO_3^- to N_2 through the above steps, denitrification in the aquatic environment is restricted to zones of reduced oxygen status, particularly sediments (Seitzinger, 1988). Sediments represent the main site for decomposition of organic matter that leads to the release of NH_4^+. The NH_4^+ becomes available for nitrification, which in turn generates NO_3^- for denitrification (Fig. 1.1). Based on evidence from concentration profiles, Billen & Vanderborght (1978) suggested that the reduction processes of various electron acceptors are separated from each other in individual sediment layers. Electron acceptors are used preferentially according to their free energy and are therefore consumed in the sequence shown below (Kerner, 1993).

$$O_2 \Rightarrow NO_3^- \text{ or Mn (IV)} \Rightarrow \text{Fe (III)} \Rightarrow SO_4^{-2} \Rightarrow \text{fermentation}$$

Thus, the surface layer of sediment can be considered to be a zone of aerobic respiration and nitrification. According to Revsbech *et al.*(1988), the thickness of this layer, or 'oxic zone', depends on:

- The concentration of oxygen in the overlying water column.
- The level of aerobic respiration.
- The photosynthetic rate, that in turn depends on algal biomass, light and nutrient supply.

It has been suggested that denitrification in sediments is usually restricted to a well-defined thin anoxic layer immediately below the oxic zone (Christensen *et al.*, 1990; Nielsen *et al.*, 1990). This denitrification zone is restricted at its upper limit by the oxic-anoxic interface and by depths of NO_3^- penetration at the lower limit, as well as by rate of NO_3^- consumption. Christensen *et al.* (1989) found that the thickness of this zone ranged between 0.7 mm at low concentrations of water column NO_3^- (110 μM) to 4 mm at high NO_3^- concentrations (1250 μM). The less energetically-favoured electron acceptors would then be used for anaerobic respiration at greater sediment depths where nitrate does not penetrate or nitrification occur.

There are two potential sources of nitrate for denitrification in sediments, by diffusion either down a concentration gradient from overlying water or from the surface aerobic layer where it is produced by nitrification (Fig. 1.2). These two distinct pathways are known as uncoupled and coupled denitrification respectively (Nielsen, 1992). The relative importance of these pathways mainly depends on the availability of nitrate and oxygen (Rysgaard *et al.,* 1994; Risgaard-Petersen *et al.*, 1994).

1.21 Factors affecting denitrification

A number of environmental factors affect the rate and end products of denitrification. These include: concentrations of nitrate, oxygen and organic carbon, as well as temperature, microbial numbers, pH and the presence of macrofauna. Laboratory studies have established that the most important of these are: nitrate availability, oxygen concentration, organic carbon availability and temperature (van Kessel, 1977a,b; Knowles, 1982; Groffman, 1991). These factors are generally the main determinants of the balance between denitrification, DNRA and ammonification, as well as the gaseous end-products of denitrification.

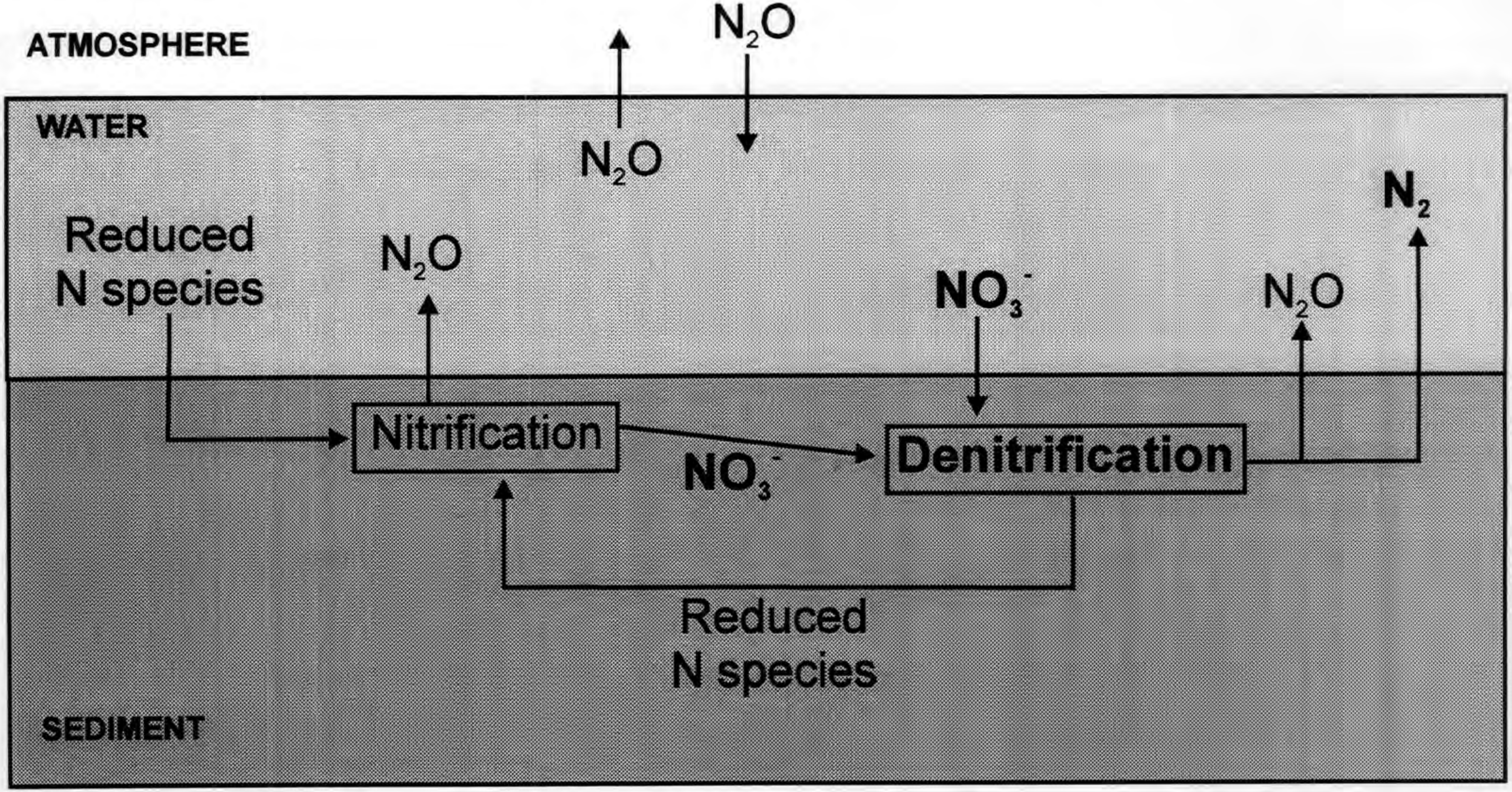


Figure 1.2 Schematic of denitrification in the aquatic environment

Nitrate

As previously discussed, denitrifying bacteria require a ready supply of nitrate to act as an electron acceptor for respiration of organic matter in anaerobic environments. Therefore, the rate at which nitrate is supplied, either from the water column or from nitrification in aerobic areas, will affect denitrification. Bowman & Focht (1974) reported that denitrification rate was affected at low ambient concentrations of nitrate, following first order kinetics, but became independent, following zero-order kinetics, at higher concentrations, possibly when other factors started to limit the rate. The diffusion rate of nitrate across the sediment-water interface is a function of the concentration gradient across the interface (Nedwell, 1982); thus the concentration of nitrate in the overlying sediment controls movement into the denitrification zone, thereby affecting denitrification. Furthermore, nitrate availability may even affect the growth rate of denitrifiers (King & Nedwell, 1987).

As well as affecting denitrification directly as a substrate, nitrate may also have a secondary influence on denitrification. Stimulation or inhibition of certain enzymes in the denitrification pathway will result in the accumulation of intermediates, thus changing the product ratio. For example, Payne (1973) describes the accumulation of NO_2^- in the presence of high levels of nitrate due to the inhibition of nitrite reductase, possibly through an indirect effect of nitrate on Eh. Nitrate has also been identified as an inhibitor of nitrous oxide reductase, resulting in nitrous oxide accumulation (Blackmer & Bremner, 1978; Tiedje *et al.*, 1981; Stewart, 1993), although some workers suggest that the NO_2^- produced may in fact be the inhibitor (Firestone *et al.*, 1979).

Oxygen

Many authors have reported that oxygen represses the rate of denitrification in soils and sediments by both direct and indirect mechanisms (Andersen, 1977; van Kessel, 1977a,b; Kaspar, 1982; Nakajima *et al.*, 1984; Dalsgaard & Revsbech, 1992). Direct mechanisms include the restriction of nitrate transport across the cytoplasmic membrane by direct inhibition of the nitrate transport proteins (Ferguson, 1988), the inhibition and repression of denitrifying enzymes (Payne, 1973), or simply by oxygen acting as the electron acceptor instead of nitrate. The supply of oxygen to the surface layer of sediment will affect the rate of nitrification, thus indirectly affecting the rate of nitrate provision to the denitrification zone beneath (Knowles, 1982). For example, Jenkins & Kemp (1984) observed a summer decrease in coupled denitrification rates compared to spring rates, possibly resulting from reduced oxygen penetration to the sediments leading to decreased nitrification.

Christensen *et al.* (1990) constructed a model which demonstrated that denitrification activity was controlled mainly by the thickness of the surface oxic layer that served as a diffusion barrier for nitrate transport to the denitrification zone from the water column. Rysgaard *et al.* (1994) concluded that in waters with low nitrate, oxygen caused an increase in denitrification as a result of increased nitrification, yet in waters with high nitrate concentrations, increased oxygen resulted in a decrease in denitrification as a result of the increase in diffusion path for intrusive nitrate down to the anaerobic denitrification zone.

Some authors have postulated that denitrification could be active in some oxic water columns (Gundersen, 1981), possibly as a result of the existence of anoxic microzones and oxygen microgradients in suspended particulate matter (Alldredge & Cohen, 1987). Jenkins & Kemp (1984) also suggested that denitrification in the upper oxic layer of sediments may be possible as a result of coupling with nitrification in sediment microparticles. More recently, it has been suggested that some bacteria are capable of aerobic denitrification. These ‘aerobic denitrifiers’ are capable of simultaneously using oxygen and nitrate as terminal electron acceptors (Robertson & Kuenen, 1984; Lloyd *et al.*, 1987; Carter *et al.*, 1995).

Similarly to nitrate, oxygen has been shown to affect the balance of gaseous end products. For example, in anoxic soil, nitrate and nitrite reductases undergo derepression before the nitrous oxide reductase enzyme; so N_2O becomes the major product. Nitrous oxide reductase then becomes derepressed and N_2 again becomes the dominant product (Firestone & Tiedje, 1979; Smith & Tiedje, 1979). Firestone *et al.* (1979) found that adding a small amount of oxygen to soil slurries greatly decreased denitrification activity, while increasing the N_2O:N_2 ratio in the products.

Organic carbon

As most denitrifying bacteria are chemo-organotrophic, the reducing power of organic carbon compounds is one of the most important factors controlling their activity. The carbon supply influences denitrification directly by supplying the necessary substrate for growth, and indirectly through the consumption of oxygen by other micro-organisms in soil and sediment (Rolston, 1981).

Denitrifying activity has been shown to be related to organic C contents in both sediments and soils (Knowles, 1982). Van Kessel (1978) found good correlation between denitrification and organic matter availability in two sediment-water systems. Various studies have also shown that slow rates of denitrification resulted directly from organic C limitation (McCarty & Bremner, 1992; Ambus, 1993). Caffrey *et al.* (1993) added organic matter to marine sediment mesocosms and found that high nitrate and organic C concentrations resulted in increased rates of denitrification. However, low nitrate concentrations with organic C additions resulted in a decrease in denitrification, possibly as a result of a decrease in depth of oxygen penetration resulting in a decrease in nitrate supply from nitrification.

Macrophytes can also affect denitrification rates (Christensen & Sørensen, 1986; Blackburn *et al.*, 1994; Nedwell *et al.*, 1994); this is presumably because their roots exude organic compounds that can be oxidised by denitrifiers.

Temperature

Temperature can directly affect denitrifying bacteria by increasing their enzyme activity and hence rate of denitrification. Indirectly, it can influence the depth of the oxidised layer in which nitrification can occur (Billen, 1982). Oxygen solubility increases with decreasing temperature, thus at lower temperatures the oxic layer will be pushed deeper (Horrigan & Capone, 1985). This may in turn increase nitrate production by increasing the area available for nitrification, yet it might also decrease denitrification rate by introducing a longer diffusion path for intrusive nitrate. Temperature may also lead to the selection of different microbial communities (Kaplan *et al.*, 1977; King & Nedwell, 1984).

Temperature also affects the balance of gaseous end products of denitrification; for example observations of an increased mole fraction of N_2O in gaseous products of denitrification with increased temperature have been made (Knowles, 1982).

Other factors

Denitrification is most rapid in neutral to slightly alkaline conditions between pH 7 to 8 (Knowles, 1982). Sprent (1987) observed that denitrification is actually suppressed below pH 3.5. The final products are also affected by pH: between pH 3.5 and 7 accumulation of nitrogen oxides may occur as denitrification may stop at NO or N_2O as a consequence of inhibition of the reductases (Bryan, 1981; Knowles, 1982). There may also be differences in species of denitrifiers at low pH (Focht & Verstraete, 1977).

Denitrification might also be affected by sediment infauna that may alter the rate of supply of nitrate, oxygen and organic matter to the denitrification zone through their burrowing activity (Christensen *et al.*, 1987).

Sørensen *et al.* (1980) found that sulphide caused partial inhibition of NO reduction and strong inhibition of nitrous oxide reductase, thus causing accumulation of nitrogen oxides. Marine sediments in particular may exhibit high N_2O production because they often contain high concentrations of sulphide as a result of bacteria using sulphate as a terminal electron acceptor.

1.22 Denitrification in riverine systems

As the first aquatic systems to receive nitrogen from N-fertilised terrestrial ecosystems, rivers can act as regulators of the flux of nitrogenous compounds to the estuarine and coastal marine environments. However, despite this potentially important role, relatively few studies have been made on denitrification in streams and rivers when compared to the marine environment (Seitzinger, 1988).

It is also clear from mass balance calculations that net nitrogen losses do occur within riverine systems (Kaushik & Robinson, 1976; Hill, 1981; Cooper, 1990). However, studies on sediment denitrification in lotic systems have focussed on relatively short stretches of stream or river, such as agriculturally-influenced streams (Cooke & White, 1987; Jansson *et al.*, 1994), a nitrate-limited stream (Holmes *et al.*, 1996) and a nitrate-enriched river (Pfenning & McMahon, 1996). None of these studies dealt with possible changes in denitrification on passing down a complete river system; this is surprising in view of the interest in downstream changes in ecological processes stimulated by the river continuum concept of Vannote *et al.* (1980).

According to the river continuum concept, the physical and chemical variables of water and sediments should present a gradient from headwaters to estuary that should in turn elicit a biological response, for example within the denitrifying bacterial population. As previously mentioned (Section 1.21), laboratory studies have established that optimal conditions for denitrification include: high nitrate supply, sufficient amounts of reducible organic substances, low oxygen concentration and high temperatures (van Kessel, 1977a, b; Knowles, 1982). In the complex riverine environment, denitrification conditions can change from near optimal to very unsuitable in short distances of space and time. This is because although the nitrate supply is usually continuous, reduced organogenic sediments are less frequent because of their enhanced oxygenation by water turbulence. Additionally, sedimentation of organic material is highly variable depending on runoff conditions and the shape of the river (Jansson *et al.*, 1994).

All the environmental influencing factors are likely to change, sometimes markedly, from the headwaters on passing downstream; thus not only may denitrification be expected to change spatially down a river continuum, but also seasonally, with changes in these factors.

1.3 Nitrous oxide

In addition to its potentially ameliorative effects regarding eutrophication, denitrification can also have potential deleterious effects through its contribution of nitrous oxide (N_2O) to the atmosphere. Nitrous oxide has a long decay time in the atmosphere (150 years to reach 37% of the original level) and contributes a large radiative forcing effect per molecule, about 200 times that of CO_2 (Rodhe, 1990). Nitrous oxide is therefore considered to be a major 'greenhouse gas' (IPCC, 1996). In addition, it participates, through its oxidation to NO, in the stratospheric destruction of the ozone layer (Cicerone, 1987).

Atmospheric N_2O concentration has increased from about 275 ppbv in pre-industrial times to about 311 ppbv in 1992, with an annual increase during the 1980s of 0.8 ppbv, perhaps falling slightly to about 0.5 ppbv y^{-1} in 1993 (IPCC, 1996). Despite the difficulty in quantifying its sources (Knowles, 1982), it is estimated that natural (and semi-natural) sources of N_2O are probably twice as large those directly linked to anthropogenic origins; furthermore, terrestrial sites have generally received more attention. Three major biological sources that may yield N_2O have been identified. As previously discussed, N_2O can be an intermediate or an end-product of denitrification (Payne, 1981) or a by-product of nitrification (Goreau *et al.*, 1980). It can also result from the dissimilatory reduction of NO_3^- to NH_4^+ (DNRA), where N_2O may be produced as a by-product (Smith & Zimmerman, 1981). Denitrification and nitrification appear to be the dominant sources of N_2O in most natural systems and denitrification is also generally recognised as the only significant means of biological removal of N_2O.

As previously discussed (see Section 1.11), the massive use of nitrogen-based fertiliser, increases in wastewater and atmospheric deposition have all increased the combined inorganic nitrogen in many rivers. Consequently, this may accelerate the production of N_2O. Based on supersaturation levels of N_2O in water, McElroy *et al.* (1978) and Kaplan *et al.* (1978) concluded that rivers may represent a significant source for N_2O, yet data for non-tidal rivers are largely lacking.

There has been a considerable number of studies made on the influence of environmental factors on denitrification (Seitzinger, 1988; Firestone & Davidson, 1989), but fewer on N_2O production. Firestone *et al.* (1979) reported that an increase in nutrient loading in soils increased N_2O production and this was confirmed for N_2O fluxes in marine sediments by Seitzinger *et al.* (1983). The latter authors reported that, during the summer, benthic N_2O fluxes in Narragansett Bay, USA, were 1.48 μmol N_2O m^{-2} h^{-1} for the eutrophic upper bay and 0.24 and 0.078 μmol N_2O m^{-2} h^{-1}, respectively, for the relatively unpolluted mid and lower bay sediments. Furthermore, Law *et al.* (1991) found that incubation of sediment from the River Tamar estuary, S-W. England, with various concentrations of NO_3^- in the overlying water (300-1500 μM), resulted in a proportional increase in N_2O production from the sediment.

1.4 Aims

Background

With the increase in nitrogen loading in surface waters in recent decades, studies have shown the potential environmental problems that can arise as a consequence of excessive nitrate in aquatic systems. To alleviate such problems, it is therefore important to understand the processes that lead to the reduction of nitrate in aquatic systems.

Denitrification is a significant biological process that can remove nitrate from an aquatic system as a gaseous product and not merely transfer it to another part of the system, for example, via macrophytic uptake. However, as observed by Seitzinger (1988), relatively few studies have been made of this process in streams and rivers compared to the marine environment. In addition, despite the fact that McElroy *et al.* (1978) and Kaplan *et al.* (1978) suggested that rivers may be a significant source of nitrous oxide, data on this for non-tidal rivers appear, once again, to be largely lacking.

Hypotheses

As discussed above, denitrification is affected by various environmental factors and, according to the river continuum concept, rivers show a gradient of such factors. The present investigation was planned in order to test the following hypotheses:

1) That a gradient of physical and chemical variables along a river continuum will elicit a graded, spatial response from denitrifying bacterial populations.

2) That physical and chemical variables along a river continuum will vary with season and will, therefore, elicit a seasonal response from denitrifying bacterial populations.

3) That certain environmental variables may exert a greater influence on denitrification rate than others.

Objectives

In order to test these hypotheses, the following objectives were devised:

1) To quantify the distribution of nitrogen compounds and other key environmental factors along a river continuum, in order to assess their relative importance spatially, and to repeat these measurements at different times so as to assess any seasonal variation in their relative importance.

2) To elucidate directly, the quantities of aquatic nitrogen being removed to the atmosphere by denitrification in sediments along a full river continuum and determine the extent of any seasonal and spatial variations in the rates of denitrification and nitrous oxide production.

3) To investigate the regulating or limiting factors, and their relative effects on denitrification and nitrous oxide production in riverine sediments.

Some studies have shown that denitrification may remove up to 53 % of nitrogen entering lake systems (Chan & Campbell, 1980; Jensen *et al.*, 1992). Therefore, by using the data collected in the present investigation it should be possible to perform a similar calculation in order to assess the significance of denitrification within a riverine environment. The data may, therefore, also help to provide a better understanding of nitrogen dynamics within a river system, and hence possibly contribute towards a more accurate model of N flux.

The objectives of the present study were addressed through both field and experimental investigations that focused on the Swale-Ouse system, together with a few comparative measurements on the River Tweed. The Swale-Ouse and Tweed river systems were chosen partly because they are the focus of a number of investigations in the LOIS programme (see Appendix 1) and partly because they are known to show consistent changes in a range of environmental variables on passing downstream (Holmes & Whitton, 1977; Robson *et al.* 1996). This, therefore, rendered them ideal for the purposes of the present study.

CHAPTER 2

STUDY AREAS AND SAMPLING PROGRAMME

2.1 Swale-Ouse river system

The Swale-Ouse system was chosen by the LOIS RACS (R) committee (see Appendix 1) for intensive study, because it is a largely unregulated system with progressive changes on passing downstream rather than a single striking change. Additionally, it is part of the Humber system, the main study area of LOIS, RACS (Fig. 2.1). The Humber system was selected for this purpose because it provides the largest contribution of freshwater to the North Sea of all British rivers. It drains over one-fifth of the land area of England (24,000 km^2), covering an area of wide diversity in terms of natural environment and land use, approximately 20% of the UK population live in this catchment area.

The River Swale (Fig. 2.2) rises on the North Pennines (N-E England) in the Yorkshire Dales National Park, running for 117 km from the confluence of Birkdale Beck and Whitsundale Beck to its confluence with the River Ure, east of Boroughbridge, after which it becomes the River Ouse. The entire Swale-Ouse system drains a catchment area of 3,200 km^2. The river changes from a neutral-acidic, oligotrophic, upland beck to a slow flowing, meso-eutrophic, lowland river.

The upper section of the Swale is steep, draining high ground, much of it Carboniferous Limestone, although Millstone Grit also occurs. The valley itself is narrow and steep-sided, so only allowing for rough grazing and resulting in the flashy nature of the river (Fig. 2.3). The river channel remains almost completely natural in formation until it reaches Richmond (the first main centre of population the river encounters); below this point significant stretches have been straightened and flood banks constructed. The river then meanders widely through the predominantly non-calcareous Vale of York, where three main tributaries join it, Bedale Beck, the River Wiske and Cod Beck, before joining the Ure. From this point the river becomes the River Ouse and flows south through the City of York to Naburn Weir, below which the river becomes tidal. The freshwater tidal reaches of the Ouse extend to downstream of Selby. The main tributaries of the Ouse are the Rivers Kyle and Foss, as well as the Nidd, Wharfe, Derwent and Aire. Other main centres of population located on this system are Catterick Garrison, Northallerton and Thirsk, although the main flood plain land use is agricultural. The rainfall for the catchment shows a wide range because the area is so large and topographically varied. At Selby the average annual rainfall is

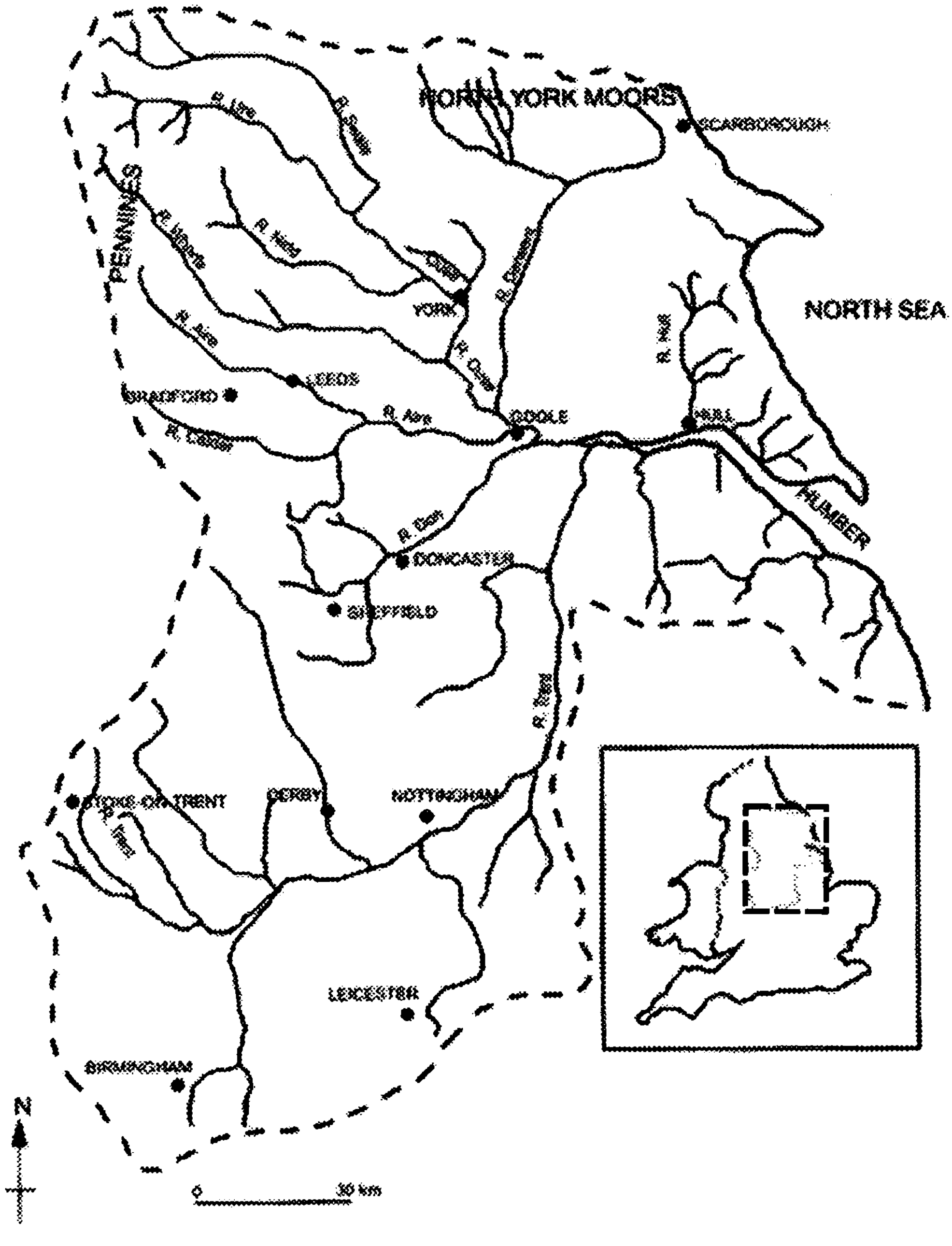


Figure 2.1 The Humber catchment

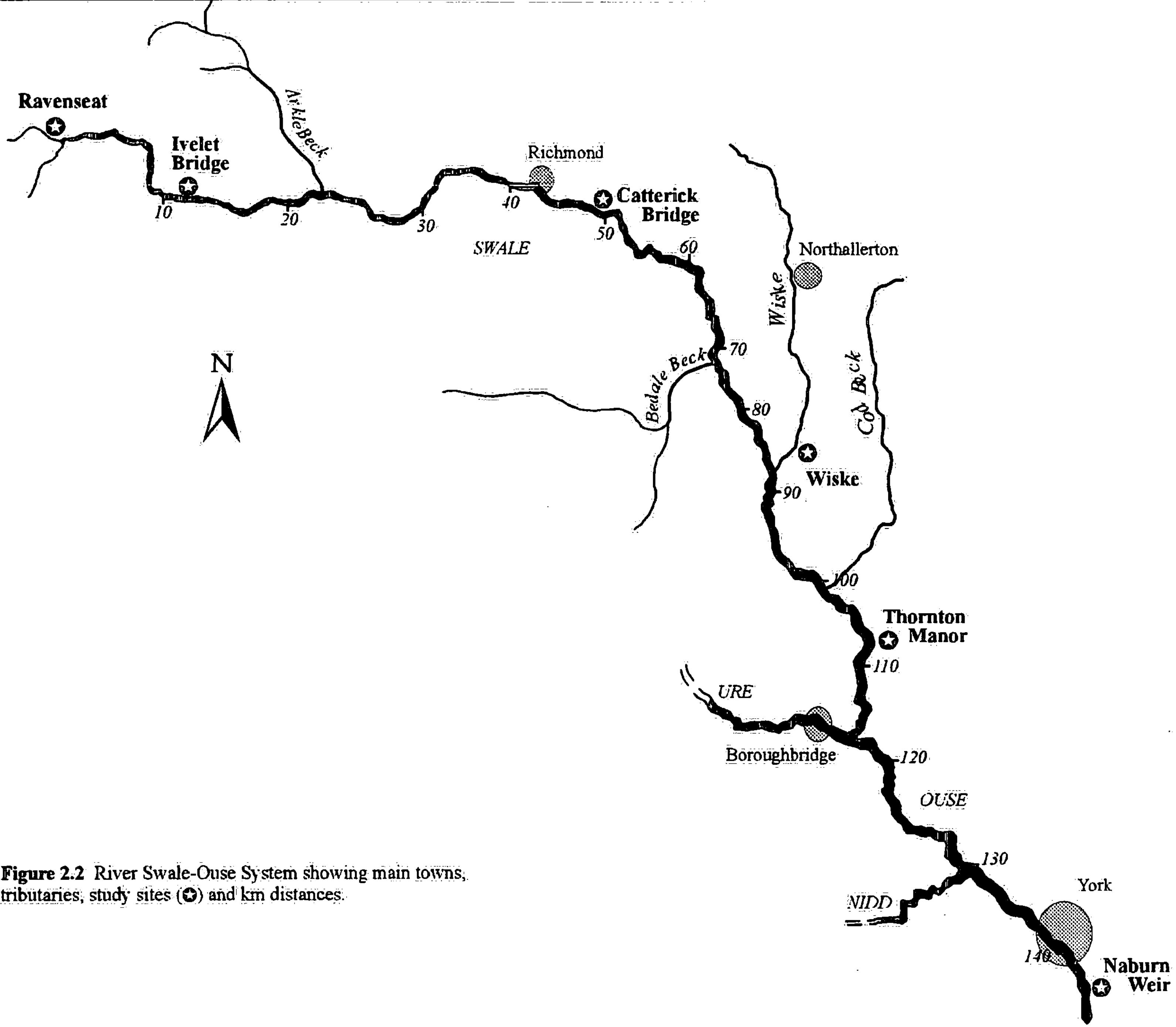


Figure 2.2 River Swale-Ouse System showing main towns, tributaries, study sites (✪) and km distances.

600 mm, whereas at the head of the catchment it is 2000 mm. A more detailed description of the geography and geology of the study area are provided by Jarvie *et al.* (1997) and Law *et al.* (1997).

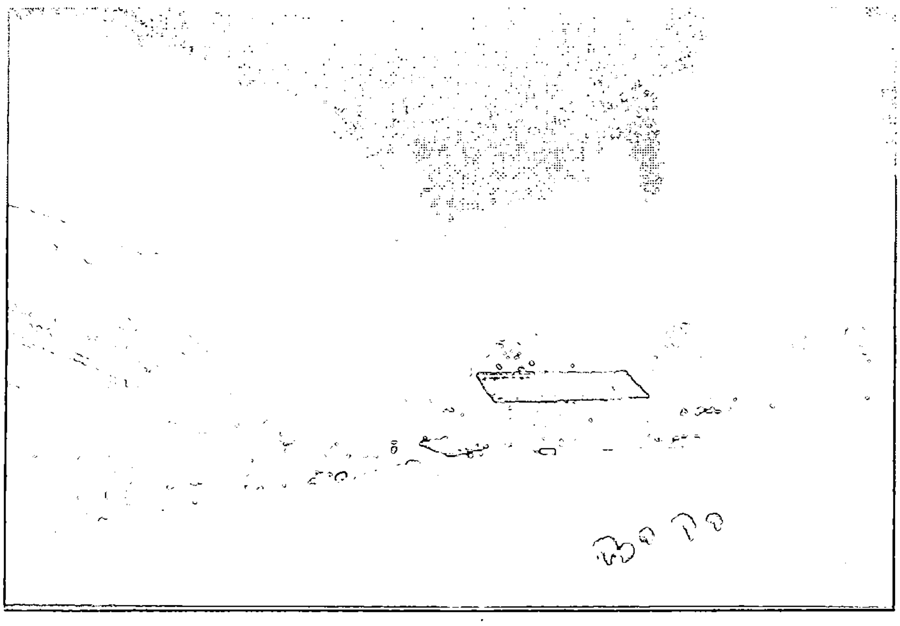


Figure 2.3 Upper Swaledale, looking down the valley (June 1996)

The chemical water quality of the Swale and its tributaries is very good (1A) on its upper and middle reaches, on its lower reaches and along the Ouse it is good (1B), although the Wiske is notable in its poor (3) quality. The downgrading of the Wiske water quality is caused by diffuse and point source pollution from rural land use, and discharges from Northallerton and Romanby sewage treatment works (Environment Agency, 1997). The water quality downstream of York and in the tidal reaches is fair or poor, this is due to the impact of urban drainage, combined sewer overflows, the River Foss and Naburn sewage treatment works. Further sewage and trade effluent discharges occur at Selby. An important source of metals in particulate form in this system is from the erosion of spoil heaps of abandoned lead-zinc mines in the Pennines (Neal *et al.*, 1997).

The biological water quality of the upper and middle reaches of the Swale is very good (B1A) and good (B1B) in the lower reaches (NRA, 1994). Biological sampling, however, highlights tributaries of fair (B2) quality, such as Whitsundale and Birkdale, which have faunas indicative of acid conditions. The Ouse to Naburn Weir varies between good and fair (NRA, 1994). There are healthy fisheries located in this system. The fish populations of the upper Swale consist mainly of native brown trout.

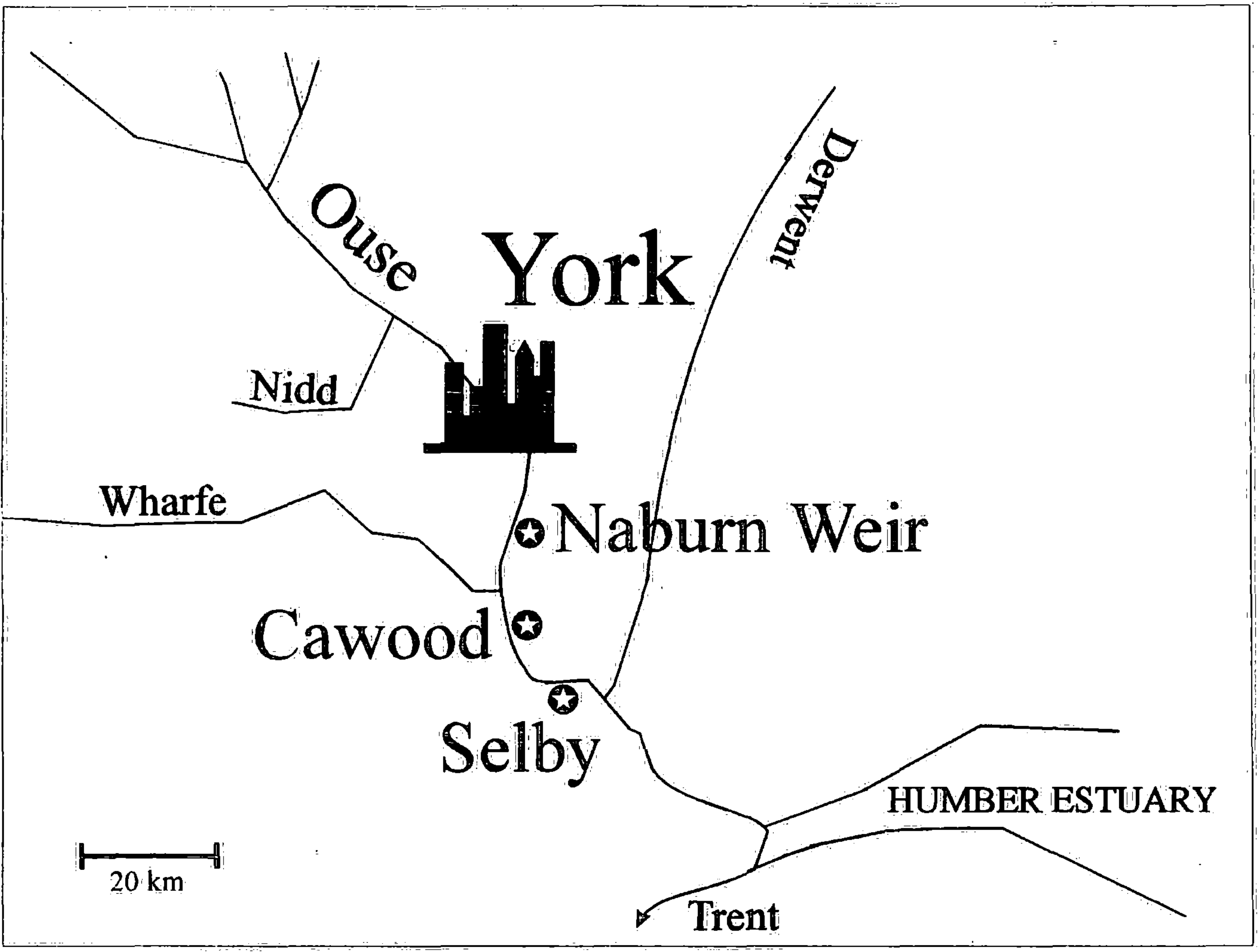


Figure 2.4 Location of sample sites in the freshwater intertidal zone of the Yorkshire Ouse

Coarse fish do not occur in great numbers until below Richmond (Environment Agency, 1997). A more detailed description of the biology of the study area is provided by Whitton & Lucas (1997).

2.11 Study sites

For the purposes of the main study, five sites were selected along the Swale-Ouse system from its upper reaches to its tidal limits to illustrate the continuum (Fig. 2.2). A sixth site on the river Wiske, a eutrophic tributary to the Swale, was added to provide the full range of trophic conditions. The sites (Table 2.1) are characterised by their river distance from the start of the main river (km 0.0), using the km distances given by Holmes & Whitton (1977); sites on tributaries are characterised by their distance upstream from the main river (indicated by a negative number). The most upstream site (km -2.5, 0.0) was located up Whitsundale Beck, one of the two tributaries which form the main river. The most downstream site (km 145.0) was located at the tidal limits at Naburn Weir. They were chosen for their compatibility with other collaborative studies involved in LOIS, such as IFE (Wareham), and the core sampling programme, as well as ease of access. Two additional sample sites for a supplementary study were located on the freshwater tidal zone downstream of Naburn Weir to Selby (Fig. 2.4). Brief descriptions of these sites are also available in Table 2.1. Additionally, to gain an impression of the varying nature of the river from upstream to downstream, photographs of some of the sites are provided (Figs. 2.5 to 2.10).

Figure 2.5 Ravenseat (km -2.5, 0.0) looking upstream (June 1994)

Table 2.1 Descriptive characteristics of the study sites in the Swale-Ouse system. Distance from the source shown in km (negative numbers indicate distance up a tributary from the confluence with the main river, see Section 2.11).

Site name	Distance (km)	Grid ref.	River/ Tributary	Elevation (m)	Width (m)	Description
Ravenseat	-2.5, 0.0	NY863032	Whitsundale Beck	400	5	Boulders, cobbles, some sand, no trees
Ivelet Bridge	10.9	SD933977	Swale	230	10	Boulders, cobbles, interstitial sand + banks, marginal shade
Catterick Bridge (*)	49.9	SE227994	Swale	180	20	Boulders, cobbles, sand bar, little marginal shade
Thornton Manor (*)	107.9	SE433715	Swale	5	15	Boulders, cobbles, sand bank
Naburn Weir (*)	145.0	SE594445	Ouse	5	30	Sand banks, > wading depth
Castle Farm	-1.7, 86.1	SE376847	Wiske	5	4	Sand, silt, clay, marginal shade
Cawood	210	SE575378	Ouse	<5	30	Sand, silt, > wading depth, tidal
Selby	230	SE627329	Ouse	<5	30	Sand, silt, > wading depth, tidal

(* indicates a core LOIS site)

Figure 2.6 Ivelet Bridge (km 10.9) looking upstream (June 1996)

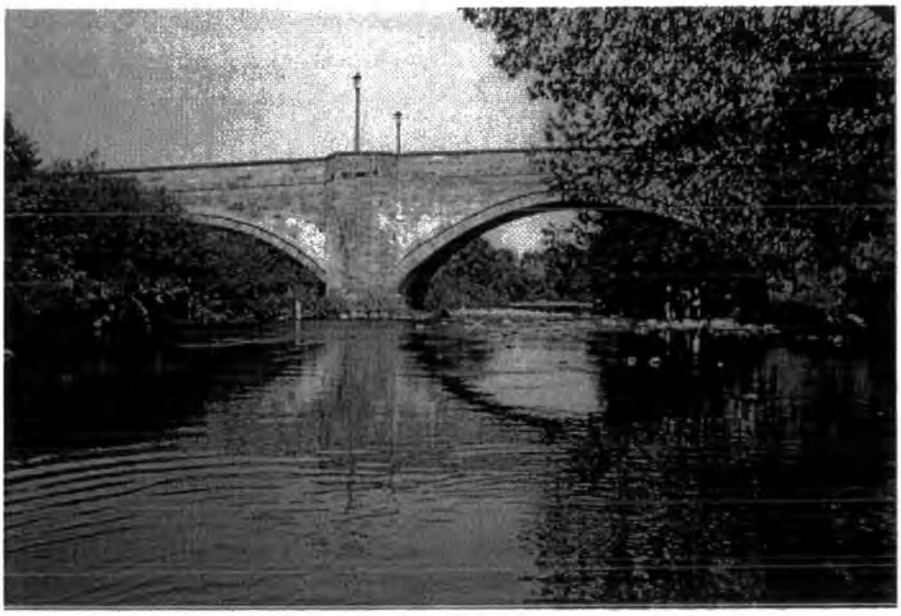


Figure 2.7 Catterick Bridge (km 49.9) looking downstream (June 1996)

Figure 2.8 Thornton Manor (km 107.9) looking downstream (July 1995)

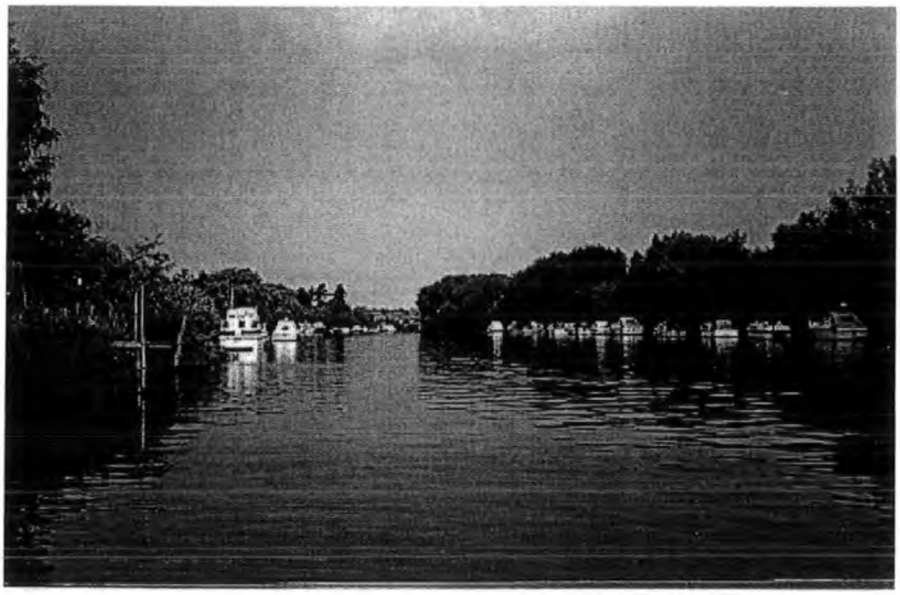


Figure 2.9 Naburn Weir (km 145.0) looking upstream from the weir (June 1995)

Figure 2.10 River Wiske (km -1.7, 86.1) looking upstream (January 1996)

2.12 Sampling programme

Water samples were taken monthly from January 1995 to December 1996 (except for km -2.5 and km 10.9 in February 1996), but with a bias introduced by the sediment sampling programme. In contrast, the LOIS core sampling was undertaken every week and also during flood events.

Sediment samples for denitrification assays were taken monthly from August 1995 to December 1996, with the exception of February 1996 when spate conditions prevented access to the main river throughout the month. Sampling at other times mostly took place in the middle of the month, with the exact date determined by flow conditions. Because of the practical difficulties in removing a core, sampling was not carried out under high flow conditions. It took two separate days to sample (and subsequently study) all sites. The dates for sampling the headwater and two most upstream sites were: 1995, 16/8; 12/9, 17/10, 14/11, 18/12; 1996, 15/1, 15/3, 18/4, 16/5, 20/6, 15/7, 16/8, 17/9, 15/10 22/11, 12/12. The remaining sites were in each case sampled two days later.

The first five months were used to develop methods and to establish that denitrification rates were easily detectable in the relatively oxic River Swale sediments. A year long study during 1996 then followed.

During the summer of 1996, from June to October inclusive, two additional sample sites were included in the monthly sampling programme, these were located on the freshwater tidal reaches of the Ouse at Cawood and Selby. Water and sediment samples were collected for Naburn Weir, Cawood and Selby on 4 July 1996, 20 August 1996 and 1 October 1996. The first sampling was carried out near low tide to provide conditions at Selby which might be expected to favour denitrification (if present) in the water column, but all the others were carried out near high tide; samples were taken on each date moving from downstream to upstream.

2.2 Tweed river system

The River Tweed was selected as a secondary study catchment, offering contrasting geomorphology and water quality characteristics to the Swale-Ouse. The Tweed is an important component of LOIS because it is one of the major UK rivers entering the North Sea. It has a large, predominantly rural, sparsely populated catchment area (4,400 km^2), draining the eastern slopes of the Scottish Southern Uplands. The whole of the Tweed and parts of some of its tributaries are notified Sites of Special Scientific Interest (SSSI).

The Tweed rises on the slopes of Broad Law and Hart Fell and flows for over 160 km to its estuary at Berwick-upon-Tweed located on the Northumbrian Coast (Fig. 2.11). There is a high proportion of upland ground within this catchment with elevations ranging from over 800 m down to sea-level. The climate is cool and temperate with an average rainfall for the catchment of 969 mm $year^{-1}$, ranging from 2200 in the headwaters to less than 650 mm $year^{-1}$ in the lowlands of Berwickshire (Fox & Johnson, 1997). The geology of the upland catchment includes a large proportion of Ordovician and Silurian greywackes, shales and mudstones overlain by peats and podzols. There are Old Red Sandstones in the lowlands, moving to Carboniferous sedimentary rocks near the coast in northern England. The upland areas comprise mainly of moorland and rough pasture and are used for hill sheep farming, whereas cereal crops can be grown in the lowlands (MLURI, 1988). Compared to the rest of the UK, industrial activity in the Tweed catchment is low and limited to a few small towns, such as Hawick, Galashiels, Selkirk and Jedburgh. Most industrial effluent is discharged to sewage for treatment or land based soakaway as opposed to direct discharges to the Tweed rivers.

The Tweed and its tributaries are mainly clean and unpolluted, supporting a diverse biology and providing an important fishing resource. Less than 1 % waters in the Tweed catchment are below class 1 status, i.e. polluted (Scottish chemical classification system; Scottish Office, 1990). Any water quality problems are relatively restricted. Eutrophication problems, such as algal blooms, occasionally arise in some of the lowland water courses draining arable areas, mainly as a result of excessive phosphorus inputs, but sometimes nitrate concentrations can also be high. Further details on the biology of the Tweed may be found in Clayton (1997) and further comments on the nature and water quality of the catchment may be found in Robson *et al.* (1996) and Robson & Neil (1997).

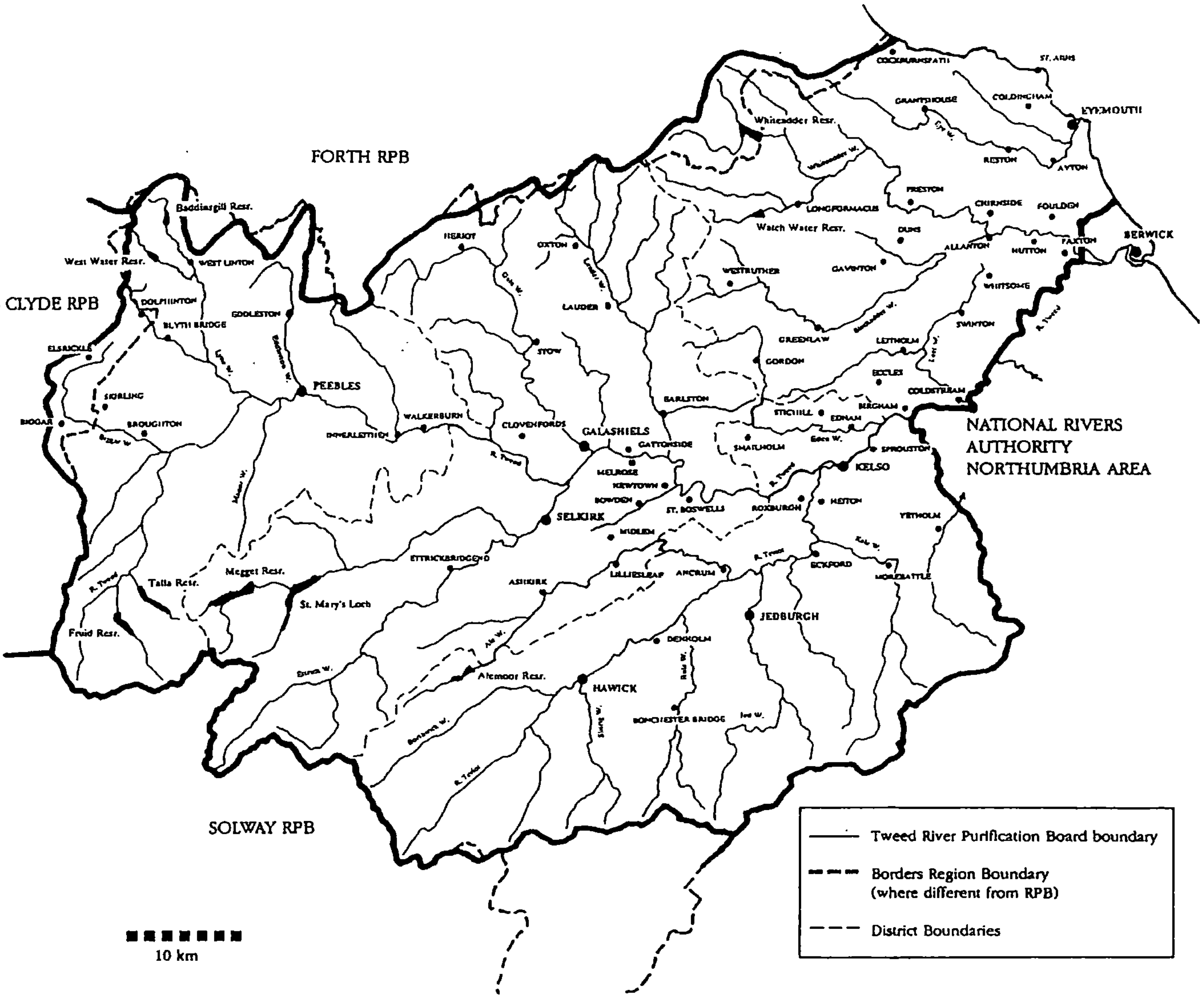


Figure 2.11 Catchment of the River Tweed

2.21 Study sites

Three study sites were selected on the main river downstream of Galashiels, a fourth site was located on the River Teviot. These sites were chosen because of their compatibility with the LOIS core programme and other Special Topic studies. No study sites were selected upstream of Galashiels because of the lack of sediment suitable for sampling for denitrification assays. The most downstream site was located in the upper freshwater tidal zone of the Tweed just upstream of Berwick-upon-Tweed. Table 2.2 provides further details on these sites.

Table 2.2 Descriptive characteristics of the study sites in the Tweed catchment. Distance from the source shown in km (negative numbers indicate distance up a tributary from the confluence with the main river; see Section 2.11).

Site name	Distance (km)	Grid ref.	River/ Tributary	Elevation (m)	Width (m)	Description
Boleside (*)	73.1	NT498335	Tweed	110	25	Boulders, cobbles, some sand
Norham (*)	138.3	NT899477	Tweed	10	30	Boulders, cobbles, some sand
East Ord	150.5	NT977518	Tweed	<10	100	Sand and some cobbles, tidal, > wading depth
Ormiston Mill (*)	-9.0, 108.5	NT705278	Teviot	50	20	Boulders, cobbles, some sand

(* indicates a core LOIS site)

2.22 Sampling programme

The four sites in the Tweed catchment were sampled seasonally (every three months) for one year from July 1996 to April 1997. The dates for sampling were: 25/6/96; 7/10/96; 3/2/97; 1/4/97. Similarly to the Swale-Ouse study, samples were collected for water chemistry and sediment denitrification when the river flows were in the lower 50 percentile and never under high flow conditions.

CHAPTER 3

MATERIALS AND METHODS

3.1 Water analysis

3.11 Collection and storage of water

Water was collected and filtered through 0.45-µm membrane filters using a Swinnex filter system. This was done in the field in order to minimise adsorption or exchange reactions that may occur with particulate material. The filtered water was transferred on ice back to the laboratory in acid washed 250-mL polyethylene screw-cap bottles and stored at 4°C in the dark and analysed within 24 h. If analysis did not occur within 24 h from collection, the filtered sample was frozen (-20°C) until analysis (Parsons *et al.*, 1984).

3.12 Physical variables

Variables measured on each sampling occasion were: conductivity, temperature, dissolved oxygen, pH, alkalinity, current speed and water. Observations of weather conditions were also made.

Water conductivity and temperature were measured using a Wissenschaftliche-Technische Werkstätten (WTW) meter (model FC 910). Dissolved oxygen was measured using a WTW meter (model OXI 91). The electrode required a steady water current of 15 cm s^{-1} past the membrane, so the electrode was stirred in the water.

A WTW meter (model pH91) was used to measure water pH, this was calibrated using standard buffers and adjusted for temperature before use. Total alkalinity was measured by titrating 50 mL of settled water sample with 0.02 M HCl until pH 4.2 was reached. The following equation according to Golterman *et al.* (1978) was used for the calculation:

$$\text{Total alkalinity (meq L}^{-1}\text{)} = v \times N \times 50000 \times 0.001639 / \mathrm{V} \qquad (1)$$

Where v is the volume of acid used, N is the normality of acid and V is the volume of sample.

Current speed was measured using a calibrated Ott meter. This was placed perpendicular to the direction of the fastest river flow. The impeller was placed $^1/_3$ the depth of the water and revolutions per minute were recorded and converted to m s^{-1}

(Patterson, 1983). Water colour was determined by absorbance at 320nm (Edwards & Cresser, 1987).

3.13 Standard laboratory techniques

All glassware, pipette tips and other materials used for analysis were washed in 10% sulphuric acid for no less than 20 minutes and rinsed 3 times in deionised water to ensure complete removal of all trace nutrients. Glassware was dried at 105 °C and plastics at 40 °C.

Stock solutions were made up using AnalaR chemicals weighed on a Sartorius 1474 balance, and dissolved in MilliQ water. All blanks and standards for nutrient analysis were made up in MilliQ water using BDH stock standards and treated in the same way as the samples.

3.14 Nitrite

Nitrite was analysed using a manual method involving its reaction under acidified conditions with sulphanilamide (aromatic amine) to form a diazonium salt. This quantitatively couples with N-1-naphthylethylenediamine dihydrochloride which produces a pink azo dye, this is then spectrophotometrically measured at 543 nm (Strickland & Parsons, 1967). The absorbance of this dye is proportional to the amount of nitrite in the sample, obeying Beer's Law up to about 500 $\mu g\ L^{-1}$ N. The detection limit was 1 $\mu g\ L^{-1}$ N (Stainton *et al.*, 1977).

3.15 Nitrate

Total oxidised inorganic nitrogen (TON) was analysed as nitrite after reduction by passing through a column of copperised cadmium filings (Strickland & Parsons, 1967). Nitrate could then be calculated by subtracting nitrite from TON.

3.16 Ammonium

Ammonium was analysed manually after Solorzano (1969). Phenol and hypochlorite reacted with ammonium under alkaline conditions to form indophenol blue. Nitroprusside was used as a catalyst to facilitate colour development at room temperature. The colour intensity, measured at 640 nm, was proportional to the ammonium concentration. The detection limit was 2 $\mu g\ L^{-1}$ N.

3.17 Verification of N fraction analysis

Inter-laboratory calibration

An inter-laboratory calibration of nitrogen fractions was carried out between the present study, Institute of Freshwater Ecology (IFE, Wareham) and National Rivers Authority (NRA) laboratories. Water samples for analysis were collected from 13 spot sample sites located in the Swale catchment in September 1994. Measurements of NO_3^-, NO_2^- and NH_4^+ analysed separately by the IFE, NRA and this study are highly comparable (Figs. 3.1, 3.2, 3.3). There is only one major anomaly (site E2, NRA data).

Use of LOIS data

Data for nitrogen fraction determinants collected as part of the LOIS core programme were downloaded from the WIS database at the Institute of Hydrology, Wallingford. These data were used to compare with results for sites also included in the study for January 1995 to December 1996. A key reason for this was to establish how much the flow-biased sampling programme for sediments (Section 3.21) might have influenced the water chemistry results. ANOVA was used to compare log transformed nitrogen fraction data from the core programme with the data from the present study.

Nitrogen fraction data, collected during the present study and the LOIS core programme between January 1995 and December 1996, were compared at three sites: Catterick Bridge, Thornton Manor and Naburn Weir. Results of ANOVA between data sets are presented in Table 3.1. Generally there is good agreement between the two data sets. There were no significant differences between the nitrite data at the three sites. However, there were significant differences between the data sets for nitrate at Thornton Manor and ammonium was significantly different at Thornton Manor and Naburn Weir. The small differences found at these sites could be attributed to the differences in the sampling strategies behind each data set. The LOIS core sampling programme was undertaken every week and during flood events, whereas in the present study sampling was on a monthly basis and was biased to lower flow conditions (Section 2.12).

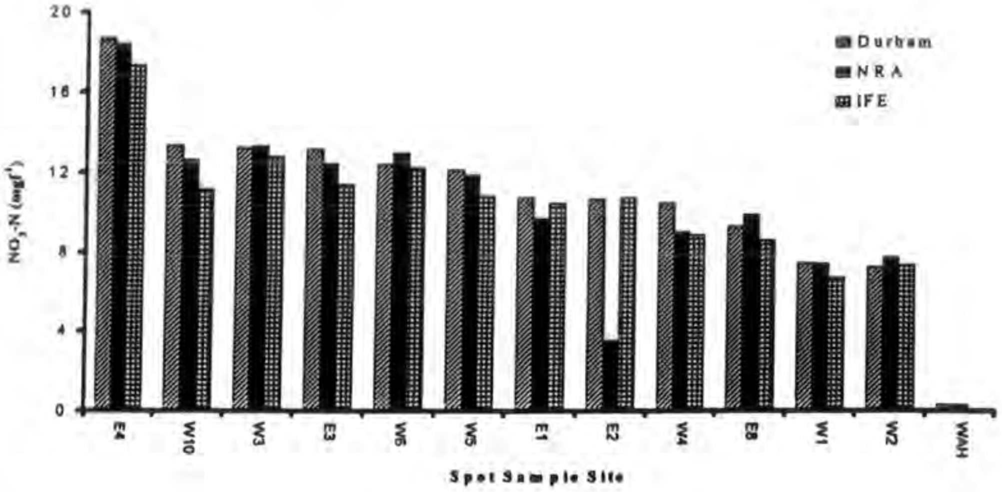


Figure 3.1 Nitrate concentration of water collected in the Swale-Ouse catchment (28/2/95). Inter-laboratory calibration of samples analysed by this study, IFE and NRA.

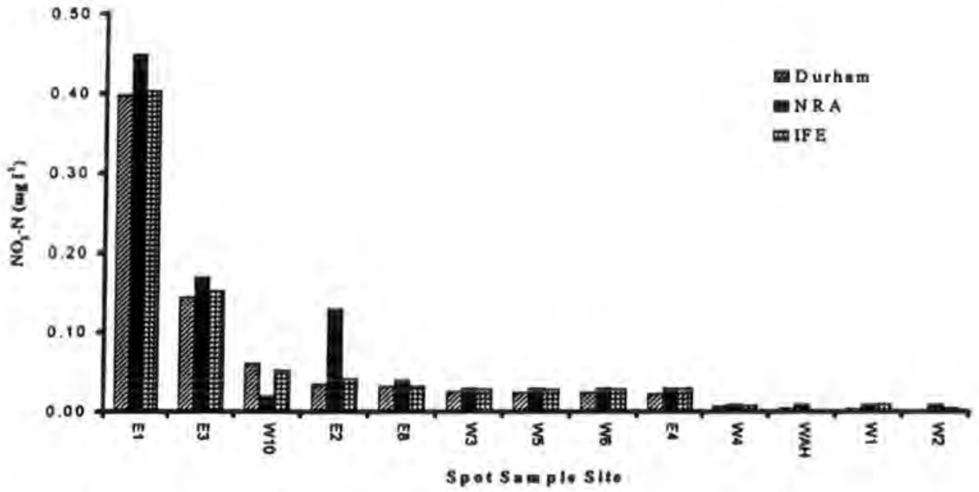


Figure 3.2 Nitrite concentration of water collected in the Swale-Ouse catchment (28/2/95). Inter-laboratory calibration of samples analysed by this study, IFE and NRA.

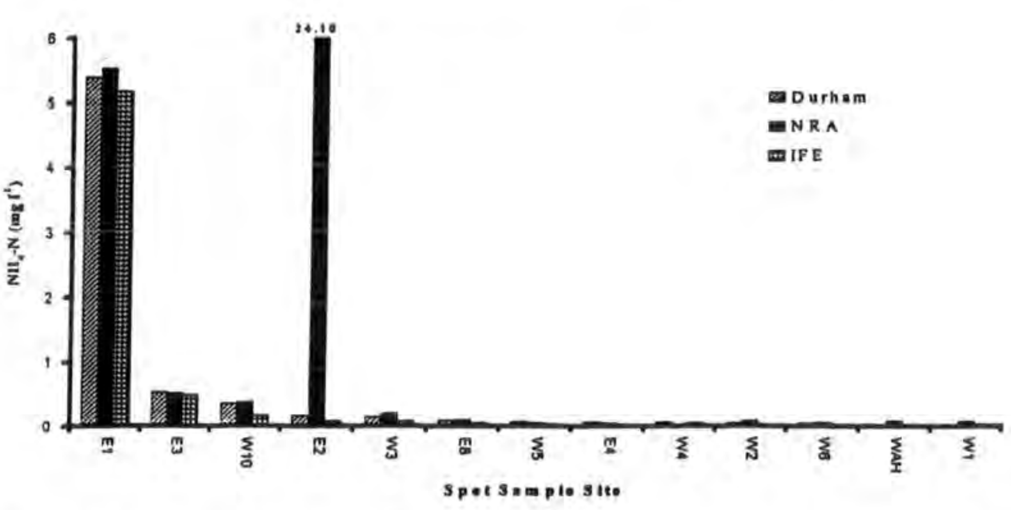


Figure 3.3 Ammonium concentration of water collected in the Swale-Ouse catchment (28/2/95). Inter-laboratory calibration of samples analysed by this study, IFE and NRA.

Table 3.1 Results of ANOVA comparing log transformed water nitrogen fraction data from the present study (monthly) and LOIS core (weekly) data. January 1995 to December 1996 (see Section 3.12 for sampling dates). (If F value < 3.92 or p-value > 0.05, then no significance difference between data sets).

Nitrogen fraction	**Catterick Bridge**		**Thornton Manor**		**Naburn Weir**	
	F value	p value	F value	p value	F value	p value
NO_3-N	2.88	0.094	7.97	0.006	0.60	0.449
NO_2-N	0.05	0.829	3.24	0.068	1.36	0.246
NH_4-N	1.29	0.258	5.67	0.019	4.40	0.038

3.18 Phosphorus

Phosphorus fractions were analysed on the day of collection. The molybdenum blue technique (Murphy & Riley, 1962) was used as modified by Eisenreich *et al.* (1975).

TFP (Total Filtrable Phosphorus) was analysed after digestion with potassium persulphate. FRP (Filtrable Reactive Phosphorus) was measured similarly but without the digestion step. The blue coloured complex that formed on analysis had an optical density of 882 nm which was proportional to the total phosphorus present. The difference between TFP and FRP was taken to be filtrable organic phosphorus (FOP). This method is applicable in a range of 1 to 500 $\mu g\ L^{-1}$ P.

3.2 Sediment analysis

3.21 Collection and storage of sediment

Intact sediment cores were taken by hand at positions in the river typically with a water depth of <1 m in defined 2 m^2 areas of sediment accumulation. The cores were collected in Plexiglas cylinders (3.5 cm inner diameter, 25 cm height) and the cylinders were sealed with a rubber bung and carefully removed from the river bed. The bottom of the cores was sealed with an additional rubber bung. Care was taken to preserve sediment structure during sampling and transport. Because of the practical difficulties in removing a core, sampling was carried out as far as possible when flow conditions were such that flows were in the lower 50 percentile and never under very high flows.

Twelve cores were taken from each site on each sampling occasion. Three sets of three cores were collected for the denitrification assay, while an additional three cores were taken for analysis of particle size, total carbon and nitrogen in the top centimetre. For potential denitrification rate assays, the top 5 cm of sediment was collected using a core and well mixed.

The denitrification assay was initiated immediately on return to the lab within 5 h of sampling (Section 3.3). The three remaining cores from each site were stored on ice in a cool box before sectioning in to 1 cm slices after the initiation of the denitrification assay. These sections were then frozen at –20 °C for storage before further analysis.

3.22 Water content

A known amount of wet sediment was dried in a crucible for 24 h at 105 °C. The sediment samples were removed from the oven and allowed to cool in a desiccator before weighing. The percentage by weight of water present in the sediment could then be calculated from the difference between wet and dried sediment.

3.23 Particle size

Particle size was determined by dry sieving. A series of five sieves was used of mesh size: 2 mm, 600 μm, 250 μm, 150 μm, 100 μm.

Approximately 20 g of dried sediment was placed on the largest sieve (2mm) of the stack. The stack of sieves was then shaken to enable the sediment to pass through the respective mesh sizes according to particle size. The percentage by weight that each fraction contributed to the whole was then calculated.

3.24 Total carbon and nitrogen analysis

Total carbon and nitrogen of dried sediment particles less than 100 μm were analysed using a CHN analyser (CARLO-ERBA EA 1108). Total carbon and nitrogen were then corrected by the percentage of particles less than 100 μm and expressed in terms of percentage weights.

Acetanilide was used as a working standard and background blanks were also run.

3.25 Nutrient extraction

The N fractions of the sediment were extracted with KCl and then determined as for water column N fractions. Approximately 3 g of moist sediment was placed in an Erlenmeyer flask with 25 mL of 1 M KCl, capped and shaken for 2 h. The samples were then centrifuged and the supernatant was analysed for nitrate and ammonium as above using KCl as a blank.

3.3 Denitrification rate measurements

3.31 Review of methodology

There are several possible methods for assessing denitrification activity: mass balance (Kaushik *et al.*, 1981; Hill, 1981); NO_3^- consumption (Andersen, 1977); ^{15}N tracer techniques (Jenkins & Kemp, 1984); direct N_2 production (Seitzinger *et al.*, 1980; Gardner *et al.*, 1987); acetylene (C_2H_2) inhibition (Sørensen, 1978a). All of these methods have their advantages and disadvantages, in particular with respect to their detection limits (Table 3.2).

Table 3.2 Detection limits in seawater of various denitrification rate measurement methodologies (Koike, 1990)

Method		**Detection limit (nmol N L^{-1})**
Colorimetric measures of NO_3^- decrease		~ 100
N_2 increase after degassing		~ 1000
^{15}N tracer methods		
	Optical emission spectrometer	~ 400
	Mass spectrometer with dual inlet	~ 10
C_2H_2 inhibition		
	Headspace method with direct injection	~ 1.5
	Cold trap concentration of N_2O	~ 0.5

Measuring NO_3^- consumption is the most simple means of measuring denitrification, however, it can not be certain that the NO_3^- has been denitrified and not just assimilated or reduced to NH_4^+. Direct measures of N_2 production require lengthy (10 days) pre-incubation, this would mean that the measured rates can not truly represent *in situ* denitrification (Rudolph *et al.*, 1991). In addition, the slightest contamination from atmospheric N_2 will result in inaccurate measures. This method also has very high detection limits.

From Koike (1990), acetylene inhibition has the highest sensitivity of all the methods and requires no nitrate additions. It is also relatively simple and inexpensive to perform (Klemedtsson *et al.*, 1990). The basic theory behind the method is that acetylene inhibits nitrous oxide reductase and the resulting accumulation of N_2O can be used as a measure of denitrification activity. Knowles (1990) observed that there are two main disadvantages of acetylene inhibition. Acetylene is also an inhibitor of nitrification (Hynes & Knowles, 1978), thus coupled denitrification may be underestimated. Kaspar (1982) observed that inhibition of N_2O reductase by acetylene may not be complete, especially if nitrate concentration is low. Both of these observations may be more of a problem in marine environments than freshwater environments. Seitzinger *et al.* (1993) suggested that acetylene inhibition underestimated uncoupled denitrification in lakes due to incomplete inhibition of nitrous oxide reductase by acetylene. However, as Ogilvie *et al.* (1997) observed, acetylene was only added to the overlying water and not also injected into the sediment, so inhibition of denitrification may indeed have been incomplete. Injection of acetylene-saturated water directly into sediment cores has been found to give realistic rates of uncoupled denitrification (Koike & Sørensen, 1988; Knowles, 1990; Raymond *et al.*, 1992). Ogilvie *et al.* (1997) also reported that acetylene inhibition was effective in their study of river Colne sediments by accounting for the NO_3^- removed by measured NH_4^+ and N_2O accumulation, as it was in a similar study by King & Nedwell (1987). Joye *et al.* (1996) also found good correlation between the acetylene inhibition method and stoichometric flux estimates of denitrification in sediments. Goulding *et al.* (1993) found no significant differences between acetylene-inhibition and ^{15}N tracer methods for denitrification estimates in soils, as did Ryden *et al.* (1979) and Mosier *et al.* (1986).

Although ^{15}N tracer methods do not have potential nitrification inhibition problems, they are not as sensitive as acetylene-inhibition. Adding $^{15}NO_3$ to the system enhances the nitrate pool, therefore kinetically stimulating the rates of nitrate uptake by denitrification and potentially resulting in overestimates. This method is also expensive and requires complicated analytical procedures (Koike, 1990). Tiedje *et al.* (1989) observed that, "Since both methods [^{15}N and acetylene techniques] are sound, it is more important to move forward and investigate underlying principles of the denitrification process than to dwell on denitrification methodology". Provided the limitations of the methods are given due consideration, these are powerful tools for quantifying the production of N_2 and N_2O from various microbial processes (Klemedtsson *et al.*, 1990).

3.32 Denitrification and nitrous oxide production

Sediment collected in the field was returned to the lab as quickly as possible for simultaneous denitrification and N_2O production rate measurements. The acetylene blockage technique (Sørensen, 1978a) was used to measure denitrification to compare with N_2O production rates.

3.321 Acetylene blockage technique

As explained in the above (Section 3.31), acetylene (C_2H_2) blocks the final reduction of N_2O to N_2 by inhibiting the enzyme nitrous oxide reductase, the consequent rate of N_2O accumulation is used as a measure of denitrification activity as it stoichometrically builds up in the place of nitrogen.

In situ measurements

This assay was initiated immediately on return to the laboratory, which was always within 5 h of sampling. Of the three triplicate sets of cores collected, the first set was used as a time zero (t_0) indication of initial N_2O concentration. The second set was used to measure N_2O production rates, and the third set was used to assess the rate of denitrification ($N_2O + N_2$) by adding acetylene to provide a final concentration of 10 %, as required to effectively inhibit denitrification and determined by Sørensen (1978a).

Water overlying all the cores was replaced with fresh site water which had been transported back to the laboratory on ice, care was taken not to disturb the surface sediment. For denitrification rate assessment cores, acetylene-saturated site water was then added to this fresh site water to obtain the required 10 % final concentration. Acetylene-saturated site water was prepared by bubbling with acetylene for 20 minutes. To eliminate any potential gaseous ammonium contamination, the acetylene was first passed through 0.1 N phosphoric acid (Sloth *et al.*, 1992). Acetylene-saturated site water was also injected into the sediment through silicon sealed septa at 1 cm intervals to achieve a 10 % saturation in the pore water as well. All cores were sealed with rubber bungs with no headspace and incubated for 4 hours (except for t_0 cores) in the dark at ambient river water temperature.

Following incubation (or immediately on sealing for t_0 cores), the cores were shaken, to equilibrate the N_2O in the sediment and water phases, and a 5 mL sample of slurry removed by syringe. This was gently transferred into a gas container, fixed with formalin (1 % v/v) and frozen at – 20°C until N_2O analysis.

After shaking the gas container for 1 minute to equilibrate gas in the headspace and sediment, 2 mL of the headspace was removed, using a gas tight syringe, for N_2O measurement. Nitrous oxide was analysed using a gas chromatograph (Perkin-Elmer 2000) equipped with a ^{63}Ni electron capture detector (ECD). Chromatographic separation was on a 4m x 1/8 inch i.d. stainless steel column packed with Porapak Q (80-100 mesh) in an oven at 60°C. Nitrogen (BOC Ltd.) was used as a carrier gas with a flow rate of 20 mL min^{-1} and the ECD temperature was 350°C. Water vapour, CO_2 and C_2H_2 all interfere with N_2O analysis using an ECD. Carbon dioxide has the same molecular weight as N_2O, so tending to coelute with N_2O and interfere with its signal (Mosier & Mack, 1980), whereas H_2O may affect chromatography by collecting in the column, and C_2H_2 reacts with the hot ECD. Quarter inch stainless steel tubes were fitted between the injection port and the column filled with 2 cm^3 $Mg(ClO_4)_2$ and 2 cm^3 14-22 mesh carbosorb to remove H_2O and CO_2 respectively. To prevent interference from C_2H_2 which eluted after N_2O, a 4-port switching valve (Valco Instruments Ltd.) was employed to vent the column elutent after the N_2O peak was resolved, a carrier gas flow was maintained over the ECD whilst the valve was switched to vent. Typical elution times for N_2O and C_2H_2 were 6 and 8 minutes, respectively. The gas chromatograph was calibrated linearly using standards made from purified N_2O (BOC Ltd.) ranging from 3.52 to 88.03 ppm. Quality control standards of 7.04 ppm were used to confirm the calibration of the gas chromatograph with every 10 sample injections.

Chromatography results were output through a Spectra-Physics SP4290 Integrator. N_2O concentrations in the cores were back-calculated from the measured headspace concentration in the gas container holding the sediment slurries using Henry's Law. The production of N_2O and denitrification rate were calculated after subtracting the initial N_2O (t_0) concentration and taking into account the incubation period and the area of the core. The N_2O production data are expressed as μmol N_2O-N m^{-2} h^{-1}, i.e. μmol N as N_2O, not N_2O. This format is used in order to permit direct comparison with results for denitrification (N_2O plus N_2), which are expressed in terms of N (again, not N_2) as μmol N m^{-2} h^{-1}.

Potential measurements

Potential denitrification was measured similarly to *in situ* rate assessments with the exception that sediment slurries in sealed 30 mL jars were used as opposed to cores. Smith *et al.* (1978) and Limmer & Steele (1982) performed similar assays using sediment slurries. Slurries were made by adding between 6-12 g of well mixed wet sediment into jars, enough to reach a depth of 1 cm, they were also sealed with no headspace following the addition of the overlying water phase. On occasions when site water was not employed as the overlying phase (for example substrate addition experiments) artificial freshwater medium without N and P was used. This was based on Chu 10 medium (Chu, 1942), a multi-purpose laboratory medium, but modified as shown in Table 3.2 (Gibson & Whitton, 1987; Grainger *et al.*, 1989). 0.25 mL L^{-1} of AC microelement stock (Kratz & Myers, 1955) (Table 3.3) was also added to the medium and buffered with HEPES (0.6 g L^{-1}) to natural pH.

Table 3.3 Artificial freshwater medium, based on Chu 10 (Chu, 1942) , a multi-purpose laboratory medium, as modified by Gibson & Whitton (1987) with AC micro element addition (Kratz & Meyers, 1955).

Chemical	**mg L^{-1}**	**µM**	**Element**	**mg L^{-1}**	**µM**
$CaCl_2.2H_2O$	35.83	243.7	Cl	20.43	576.3
$MgSO_4.7H_2O$	25.00	101.4	Ca	9.77	243.7
$NaHCO_3$	15.85	188.6	Na	4.74	198.1
KCl	4.28	57.4	S	3.26	101.7
$Na_2EDTA.2H_2O$	1.67	4.20	Mg	2.46	101.4
$FeCl_3.6H_2O$	1.21	4.50	K	2.24	57.3
			Fe	0.25	4.5
AC micro elements					
H_3BO_3	0.715	11.56	B	0.124	11.50
$ZnSO_4.7H_2O$	0.056	0.190	Zn	0.013	0.190
$MnCl_2.4H_2O$	0.045	2.280	Mn	0.012	0.218
$NiSO_4.7H_2O$	0.038	0.135	Ni	0.008	0.030
$CuSO_4.5H_2O$	0.020	0.078	Cu	0.005	0.078
$CoSO_4.7H_2O$	0.010	0.035	Mo	0.003	0.013
$Na_2MoO_4.2H_2O$	0.007	0.028	Co	0.002	0.037

On occasions when specific concentrations of nitrate were required in the medium, KCl was replaced by the required concentration of KNO_3-N. Although previous studies have apparently assessed the effects of nitrate concentration on denitrification rate by adding a range of KNO_3, an experiment was performed in order to establish that K^+ had no detectable influence. This was done by adding extra K^+ as KCl (0.5 g L^{-1}) to the lowest and highest nitrate concentrations assayed. Then denitrification was compared with those without extra K^+. No significant differences ($p<0.01$) were found when adding KCl.

N_2O production (slurry without C_2H_2) and denitrification rate (amended C_2H_2 slurry) were calculated after subtracting the initial N_2O concentration and the result expressed as nmol N_2O-N g^{-1} d.wt h^{-1} for the former and as nmol N g^{-1} d.wt h^{-1} for the latter.

3.322 Verification of denitrification methodology

A series of experiments were performed to confirm the reliability of the above methodology.

Time course

Time course experiments were performed to confirm linearity of N_2O accumulation in sediment cores over the 4-h incubation period.

Regression lines plotted on Figures 3.4 to 3.6 confirm linear production of N_2O over 5 h periods at: Thornton Manor (30/8/96); Catterick Bridge (27/8/96); River Browney (22/8/96). All the lines describe over 97 % ($p<0.001$) of the total variation in the data which is accounted for by the regression, as indicated by the coefficient of determination (R^2 values).

Sediment depth incubations

Incubations of Wiske sediment slurries taken from 0-7 cm depth at 1 cm intervals were made (2/7/96) to confirm that denitrification activity was located in the upper 3 cm as demonstrated by Jørgensen & Sørensen (1988). This was to ensure that where C_2H_2 was injected into the pore water, denitrification should be inhibited.

Figure 3.7 shows that the greatest denitrification activity is located in the upper 3 cm where C_2H_2 was injected.

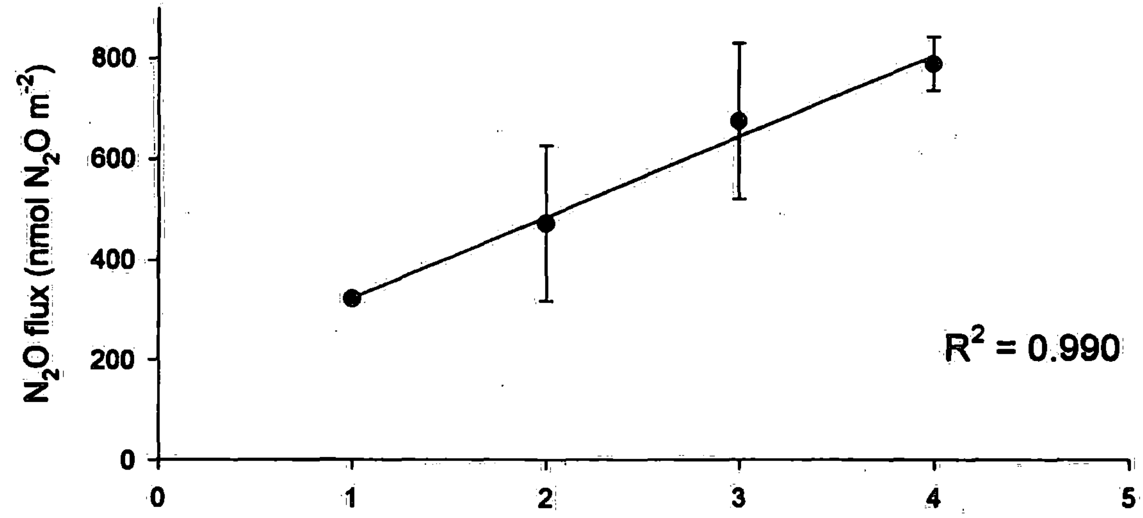


Fig. 3.4 Time course of N_2O production in sediment cores from Thornton Manor (30/8/96). Bars indicate standard error (n = 3)

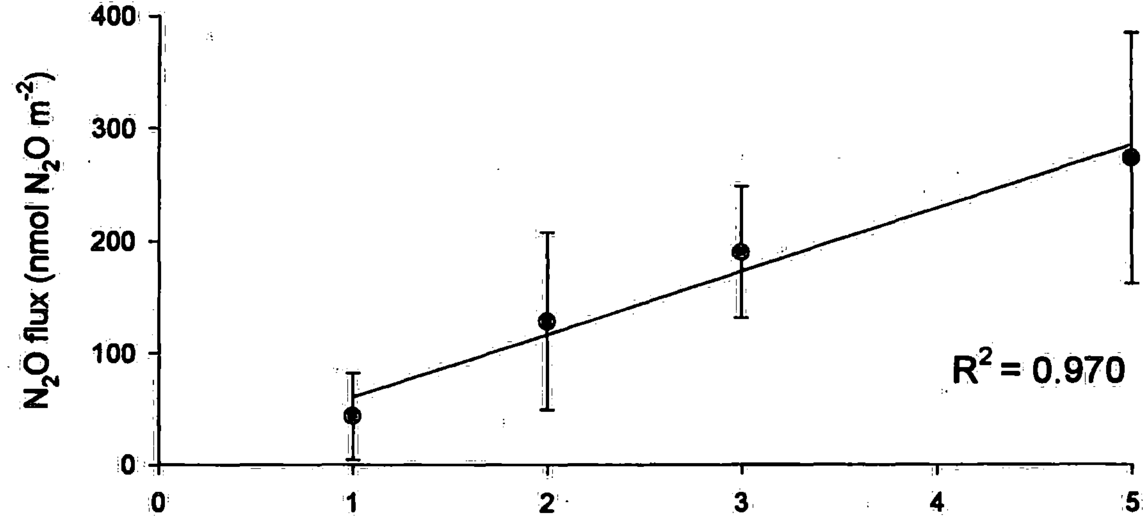


Fig. 3.5 Time course of N_2O production in sediment cores from Catterick Bridge (27/8/96). Bars indicate standard error (n = 3)

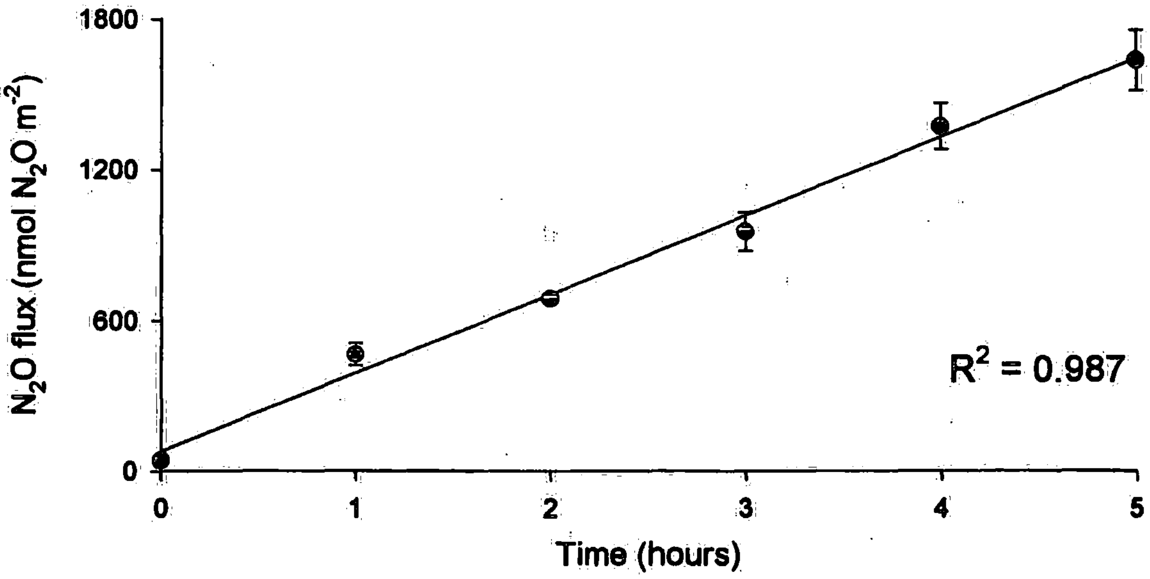


Fig. 3.6 Time course of N_2O production in sediment cores from River Browney (22/8/96). Bars indicate standard error (n = 3)

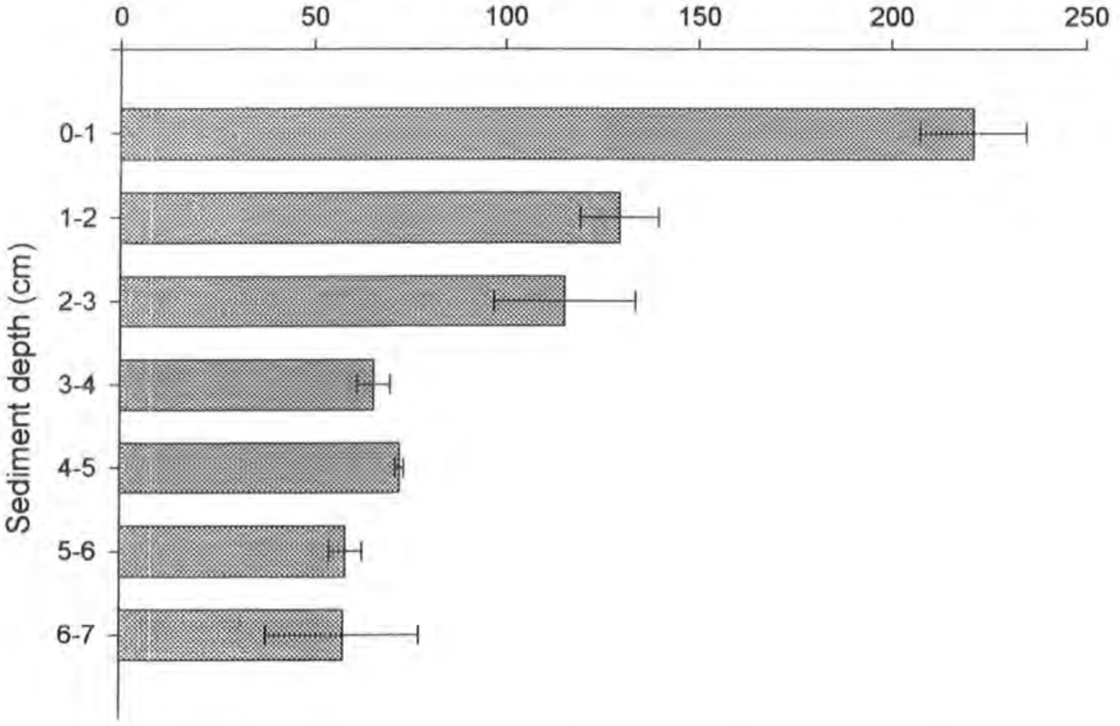


Figure 3.7 Depth profile of denitrification in Wiske sediment (2/7/96). Bars indicate Standard Error (n=3).

C_2H_2 injection

A series of incubations of cores was made to confirm that injection of C_2H_2 into the sediment was necessary to inhibit reduction of N_2O to N_2 in the sediment. Three sets of three cores were treated as described above. However, one set received no C_2H_2 in the overlying water or by sediment injection (control), a second set received C_2H_2 in the overlying water only, and C_2H_2 was injected into both the sediment and overlying water of the third set.

Figure 3.8 shows that the measured denitrification rate is approximately three times greater with C_2H_2 injection into the sediment than without injection.

C_2H_2 percentage additions

Experiments were performed to confirm that 10 % C_2H_2 was sufficient to completely block the further reduction of N_2O, as determined by Sørensen (1978a) in marine sediments. Additions of C_2H_2, ranging from 0 to 20 %, were made in sediment slurries.

Figure 3.9 shows that there is little difference in the amount of N_2O produced in sediment slurries with C_2H_2 percentage saturations ranging from 3.3 to 20 %. However, there is markedly less N_2O produced in slurries with no C_2H_2 addition.

Stirring of overlying water

An experiment was performed to ensure that stirring of the overlying water of cores was not necessary for short incubation periods of 4 h. A series of cores of sediment from the Wiske (3/6/96) were incubated as above with and without stirring. A glass stirring flea was suspended by fishing line attached to the bung; the cores were then placed in a shaking water bath which caused the flea to circulate the overlying water. Initial tests using a dye had confirmed that the fleas stirred the water sufficiently.

No differences in denitrification rates were found between stirred and unstirred cores during the 4-h incubation period (Fig 3.10).

3.323 ^{15}N-gas production

An adaptation of ^{15}N-gas flux methodology (Goering & Dugdale, 1966) as further modified by Koike (1990) was employed to verify the acetylene block methodology by comparison of the two methods. ^{15}N-gas flux methodology involves

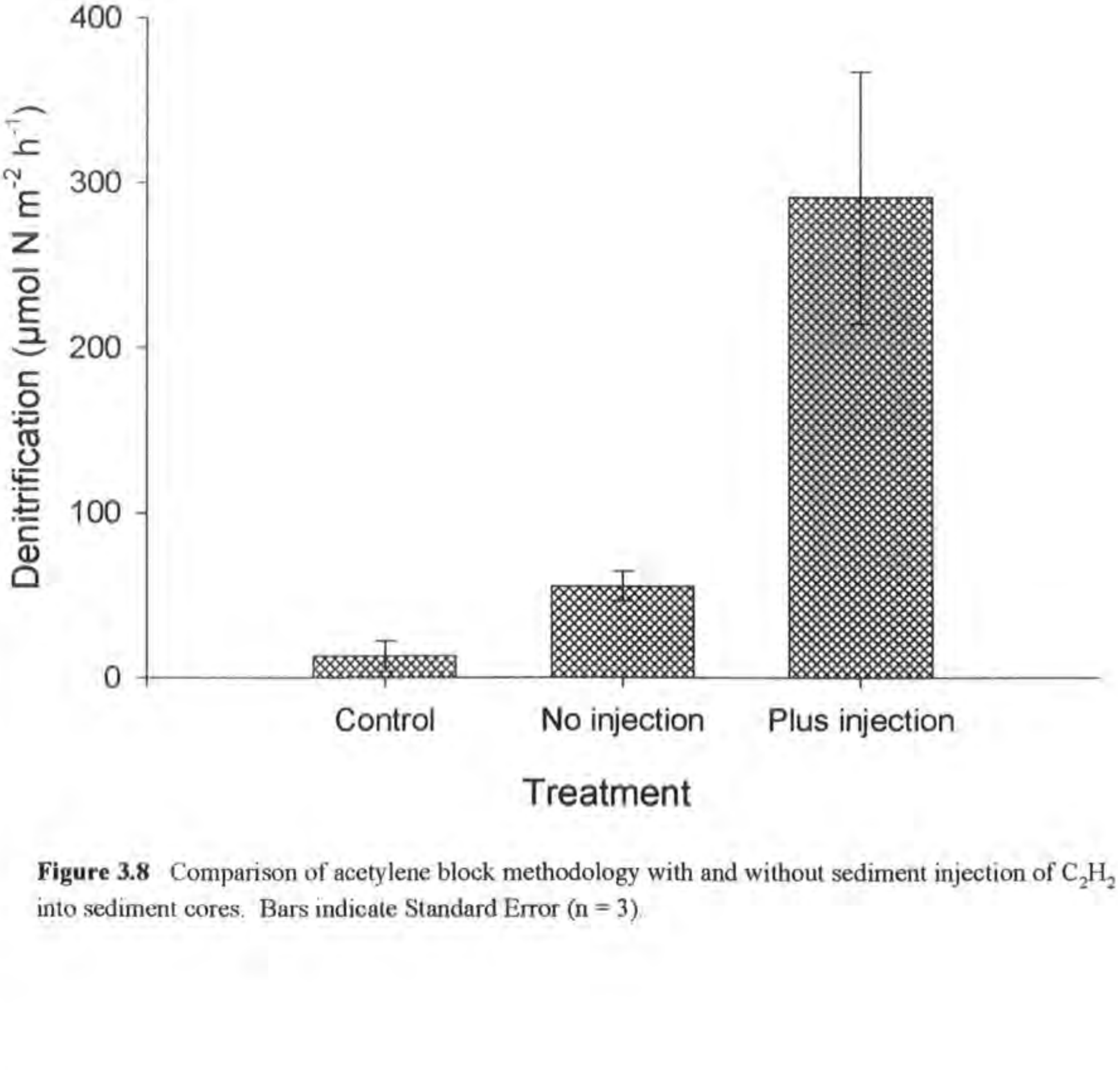


Figure 3.8 Comparison of acetylene block methodology with and without sediment injection of C_2H_2 into sediment cores. Bars indicate Standard Error (n = 3).

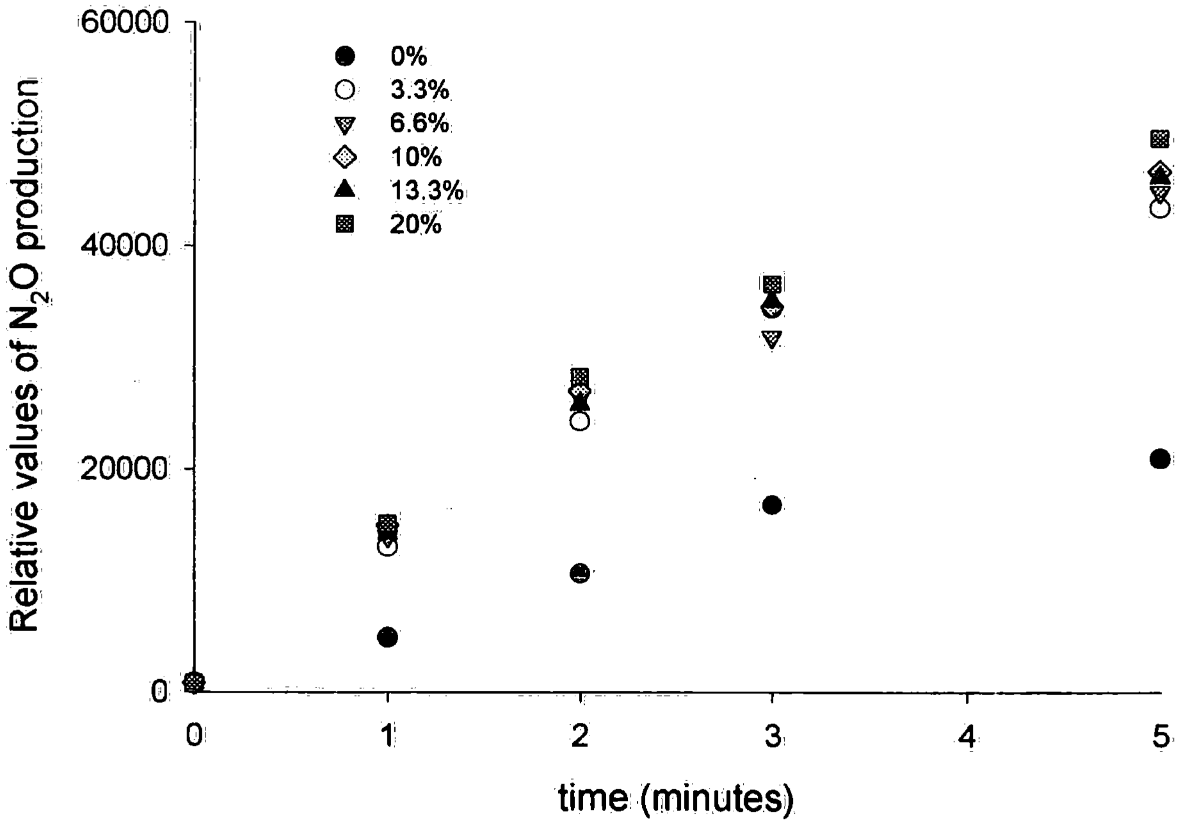


Figure 3.9 Comparison of N_2O production with different percentage additions of C_2H_2 to sediment slurries of sediment from the Wiske (31/8/96).

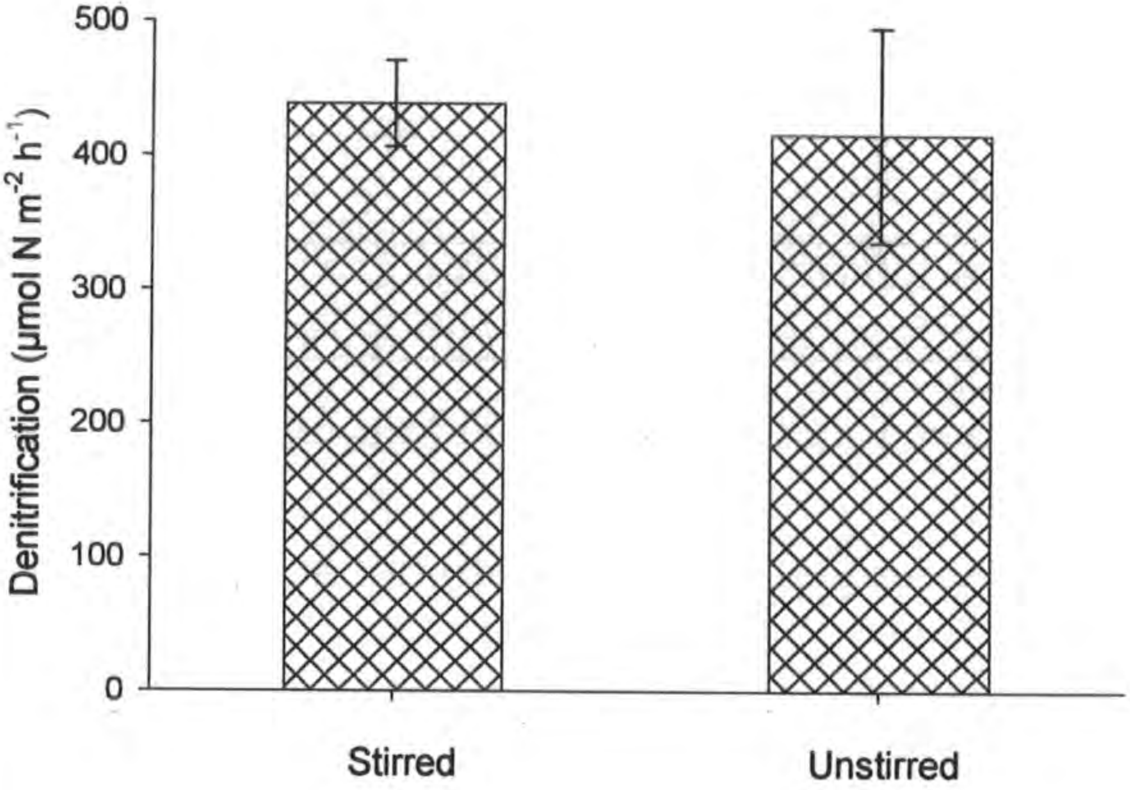


Figure 3.10 Comparison of denitrification rate in sediment cores from the Wiske with overlying water stirred and unstirred (3/6/96). Bars indicate standard error (n=3).

the introduction of a spike of ^{15}N-labelled nitrogen as nitrate and the subsequent production of ^{15}N-labelled nitrogen can then be used to calculate the rate of denitrification.

Sediment cores from the Wiske were sampled as for the acetylene block assay (3/7/96). After replacement of the overlying water with site water, four cores were spiked (in the overlying water) with $^{15}NO_3^-$ (40 atom %). These cores and four reference cores with no spike were then incubated with no headspace for 4 h, as for the acetylene blocked cores. Following incubation, the cores were then shaken and 50 mL of slurry from the core was withdrawn and shaken in a gas tight syringe with 50 mL of air for 2 min to equilibrate the gas and aqueous phases. The gas from the syringe headspace was transferred to an evacuated gas sampling tube prior to analysis.

^{15}N in the headspace gas was analysed using a Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser (at Belfast) interfaced to a Europa Scientific Trace Gas Preparation System. Details of the equations used and performance characteristics of the IRMS have been described by Stevens *et al.* (1993). The amount of ^{15}N-labelled nitrogen produced was calculated as described by Mosier & Schimel (1993) taking into account Henry's Law.

Figure 3.11 shows that in a comparison of the two methodologies using sediment from the Wiske in July 1996, the calculated average rates are very similar.

3.4 Microbiological analysis

3.41 MPN counts

Enumeration of denitrifying bacteria was performed using a modification of the Most Probable Number (MPN) method of Focht & Joseph (1973) as adapted by Tiedje (1982) and described by Martin *et al.* (1988).

Sterile test tubes with butyl rubber stoppers containing 10 mL of 5 mM KNO_3 in nutrient broth were inoculated with dilutions of sediment. These dilutions were prepared by vigorously shaking 10 g of moist sediment in 95 mL of 0.85 % saline solution with 1 drop of Tween 80 (BDH Ltd) to help the dispersion of the sediment and bacteria. A series of 10-fold dilutions from 10^{-3} to 10^{-7} was then used to inoculate five tubes with 0.1 mL for each dilution level. These tubes were then anaerobically incubated in the dark at 30°C for 14 days. After incubation, tubes with denitrifiers were identified by depletion of both NO_3^- and NO_2^- using the diphenylamine negative spot test. This involved the dropwise addition of up to 6 drops of diphenylamine

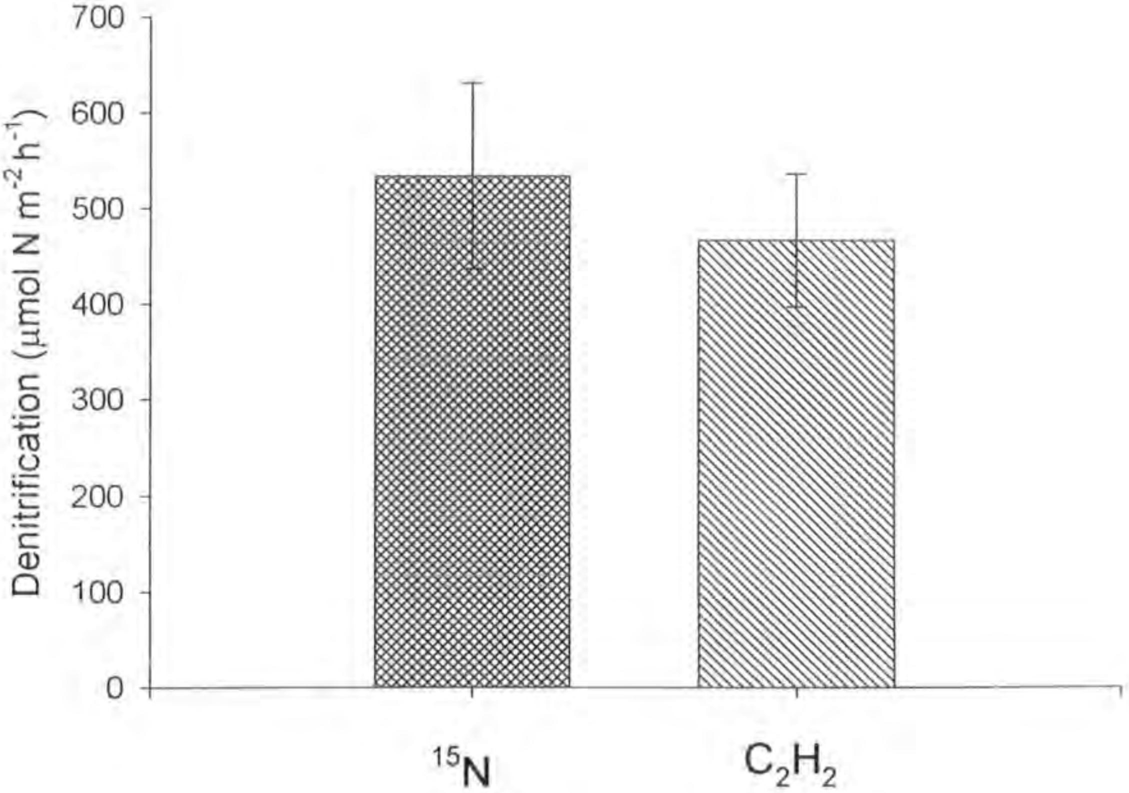


Figure 3.11 Comparison of denitrification rates using ^{15}N and acetylene block methodologies in sediment cores of the Wiske (7/3/96).

reagent to about 0.5 mL of medium from the tubes. A blue colour indicated the presence of NO_3^- and NO_2^-; a colourless response was taken as indicative of denitrifying activity. Estimates of denitrifying populations were made using an MPN table (Cochran, 1950). Denitrification was confirmed by analysing 0.5 mL of headspace from tubes aseptically injected with 1 mL of C_2H_2 following the addition of sediment dilutions for the production of N_2O using gas chromatography, as described above.

3.5 Storage of data

All the data produced from this study have been transferred to the LOIS database maintained at the Institute of Hydrology, Wallingford, for inclusion in the LOIS CD-ROM.

3.6 Computing and statistics

A pentium PC was used for the computing. MS Word97 was used for all word processing and Corel Draw was used for creating diagrams and maps. MS Excel 97 was used as a spreadsheet for data manipulation and storage, although MS Access 97 was also used for raw data storage, recovery and transfer to the LOIS database. Minitab, Release 11, for Windows was used for statistical assessments of the data and Excel 97 and Sigma Plot for Windows were both used for constructing graphs.

CHAPTER 4

SWALE-OUSE FIELD STUDY

4.1 Introduction

The aim of the study on the Swale-Ouse was to provide data to substantiate the hypothesis that a gradient of physical and chemical variables along a river continuum will elicit a graded response from denitrifying bacterial populations, not only spatially, but seasonally as well (Section 1.4). To achieve this, it was necessary to initiate a long-term sampling programme (August 1995 – December 1996; see Section 2.1) to collect denitrification and environmental data along the Swale-Ouse river system.

In particular, the seasonal and spatial trends of denitrification and N_2O production in the sediments of this system, together with relevant environmental factors, are quantified in this study. The seasonal and spatial differences in these environmental factors serve to show the changes in the nature of the system on moving downstream, from an oligotrophic upland beck to a meso-eutrophic lowland river. Thus, providing the necessary graded environment to also allow an assessment of the effects of environmental factors on denitrification. This investigation was further enhanced by a study into small-scale spatial heterogeneity, to assess the differences in rate within a site.

In order to further assess the effects of varying environmental factors on denitrification, from March to October 1996 a supplementary study extended the long-term sampling programme of the Swale-Ouse into the freshwater tidal part of the Ouse from Naburn Weir (km 145.0) down to Selby (km 230). Results from this investigation for basic environmental data and rates of denitrification measured in this reach are assessed.

4.2 Environment

4.21 Physical variables

Water temperature and flow were negatively correlated ($p<0.05$) for all the downstream sites and showed a seasonal bias (Fig. 4.1). Flow increased with distance downstream and exhibited a winter peak, decreasing in early spring through to a summer low. Flow for the tributary, River Wiske (km -1.7, 86.1,) was less than for the main river, although the same seasonality was exhibited. The temperature at the upstream sites was always lower than for the downstream sites due to their elevation.

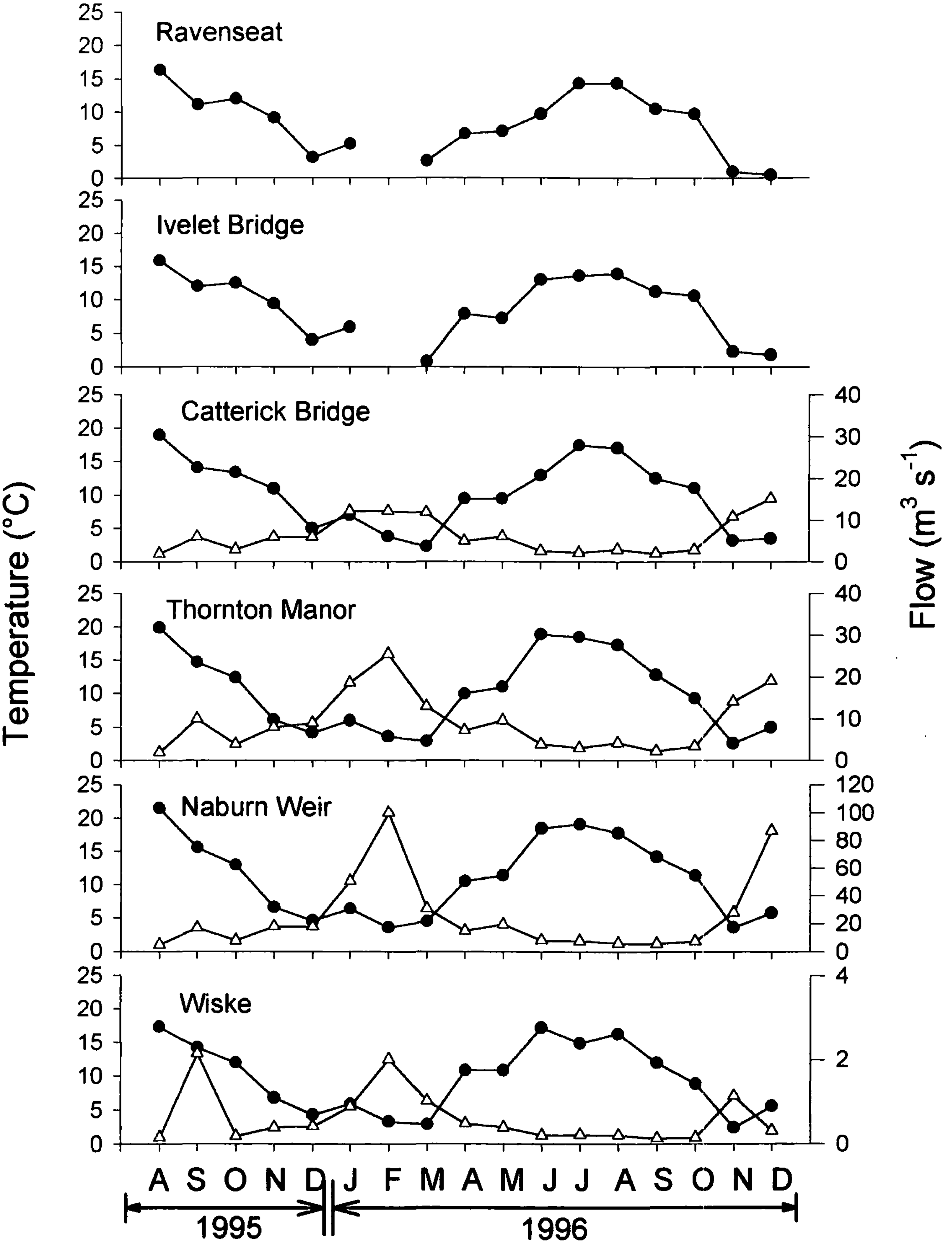


Figure 4.1 Monthly temperature (full circle) and flow measurements (open triangle) from August 1995 to December 1996. Note the different scales between sites and variables.

Seasonally, the rate of temperature increase was greatest through the spring months (March to May) reaching a summer high at all sites.

Conductivity, alkalinity and pH all increased with distance from the source (Table 4.1). The most upstream site, Ravenseat (km -2.5, 0.0), had relatively low concentrations of ions as estimated by conductivity, which never exceeded 130 μS cm^{-1}, whereas at the tidal limits at Naburn Weir it averaged 590 μS cm^{-1}. The highest conductivity values were always recorded on the Wiske, ranging from 1120 to 1557 μS cm^{-1} in 1996. There was no clear seasonality evident within the conductivity data. Alkalinity mirrored conductivity, with the lowest recorded measurements at Ravenseat, where it averaged 0.4 meq L^{-1}.

The lowest pH values were recorded at Ravenseat; the mean was 5.9, ranging from a minimum of 4.6 in December 1996 to a maximum of 7.1 in July 1996. Ivelet Bridge (km 10.9) also had a tendency to become weakly acidic in the winter months, with, for example, values of 5.9 in November 1996 and 7.9 in July 1996. The pH remained relatively stable throughout the year at around 7.5 at the lowland sites. This is a reflection of an increase in the buffering capacity of the river downstream of Richmond, due to the influence of limestone in this part of the catchment as well as the first major anthropogenic inputs from Richmond STW. Figure 4.2 highlights the strong seasonal trend in pH data at the upstream sites, Ravenseat, Ivelet Bridge and Catterick Bridge (km 49.9). A winter decrease in pH at these sites is probably indicative of the effect of increased run off from the upland acidic peat soil.

Absorbance at 320 nm has been taken as an indication of total organic carbon in the water originating from soil organic matter dissolution as water percolates through organic-rich soil horizons such as peat (Edwards & Cresser, 1987). The absorbance was greatest at the upstream sites decreasing downstream due to dilution of the colour (Table 4.1). Generally most sites showed high oxygen saturation throughout the year (Table 4.1), the Wiske was an exception with summer lows down to 46 % being recorded in July 1996. Naburn Weir also dropped below 100 % saturation in July 1996 to 75 %.

4.22 Water chemistry variables

Nitrogen fractions

Nitrate concentration in the river water gradually increased from source to tidal limits (Fig. 4.3). In the upper reaches of the catchment, concentrations rarely exceeded

Table 4.1 Means and ranges of physical water variables measured from January to December 1996 (excluding February). Abs. = Absorbance, Cond. = Conductivity; Alk. = Total alkalinity.

	RS			IB			CB			TM			NW			W		
km Distance	**0, -2.5**			**10.9**			**49.9**			**107.9**			**145**			**86.1, -1.7**		
		min.	max.		min.	max.		min.	max.		min.	max.		min.	max.		min.	max.
Abs. (320 nm)	**0.28**	0.13	0.48	**0.19**	0.09	0.36	**0.13**	0.07	0.20	**0.10**	0.04	0.14	**0.09**	0.04	0.16	**0.07**	0.05	0.08
Cond. ($\mu S\ cm^{-1}$)	**90**	56	127	**192**	100	402	**328**	180	402	**581**	411	686	**590**	384	1340	**1390**	1120	1557
O_2 (%)	**117**	107	130	**112**	99	127	**112**	101	124	**106**	95	115	**95**	75	112	**69**	46	106
O_2 ($mg\ L^{-1}$)	**13.7**	10.8	16.4	**13.0**	10.6	16.4	**12.7**	10.2	14.6	**12.0**	9.6	14.5	**10.7**	7.2	13.9	**7.7**	4.7	14.1
pH	**5.9**	4.6	7.1	**7.0**	5.9	7.9	**7.7**	6.5	8.6	**7.7**	7.0	8.4	**7.5**	6.9	7.9	**7.4**	6.9	7.7
Alk. ($meq\ L^{-1}$)	**0.4**	0.1	0.7	**1.5**	0.5	2.4	**2.1**	1.3	3.3	**3.1**	1.9	4.6	**2.6**	1.8	2.9	**4.7**	2.8	9.0

Table 4.2 Means and ranges of phosphorus fractions measured from January to December 1996 (excluding February). FRP = Filterable reactive phosphate; FTP = Filterable total phosphate; TP = Total phosphate.

	RS			IB			CB			TM			NW			W		
km Distance	**0, -2.5**			**10.9**			**49.9**			**107.9**			**145**			**86.1, -1.7**		
		min.	max.		min.	max.		min.	max.		min.	max.		min.	max.		min.	max.
FRP ($\mu g\ L^{-1}$)	**7**	1	21	**7**	1	22	**142**	40	271	**396**	128	848	**518**	156	997	**2160**	904	6316
FTP ($\mu g\ L^{-1}$)	**12**	7	20	**11**	5	23	**158**	48	284	**424**	130	983	**562**	183	1116	**2316**	1028	6155
TP ($\mu g\ L^{-1}$)	**14**	8	23	**14**	6	25	**190**	56	491	**453**	149	1032	**606**	194	1175	**2389**	1043	6380

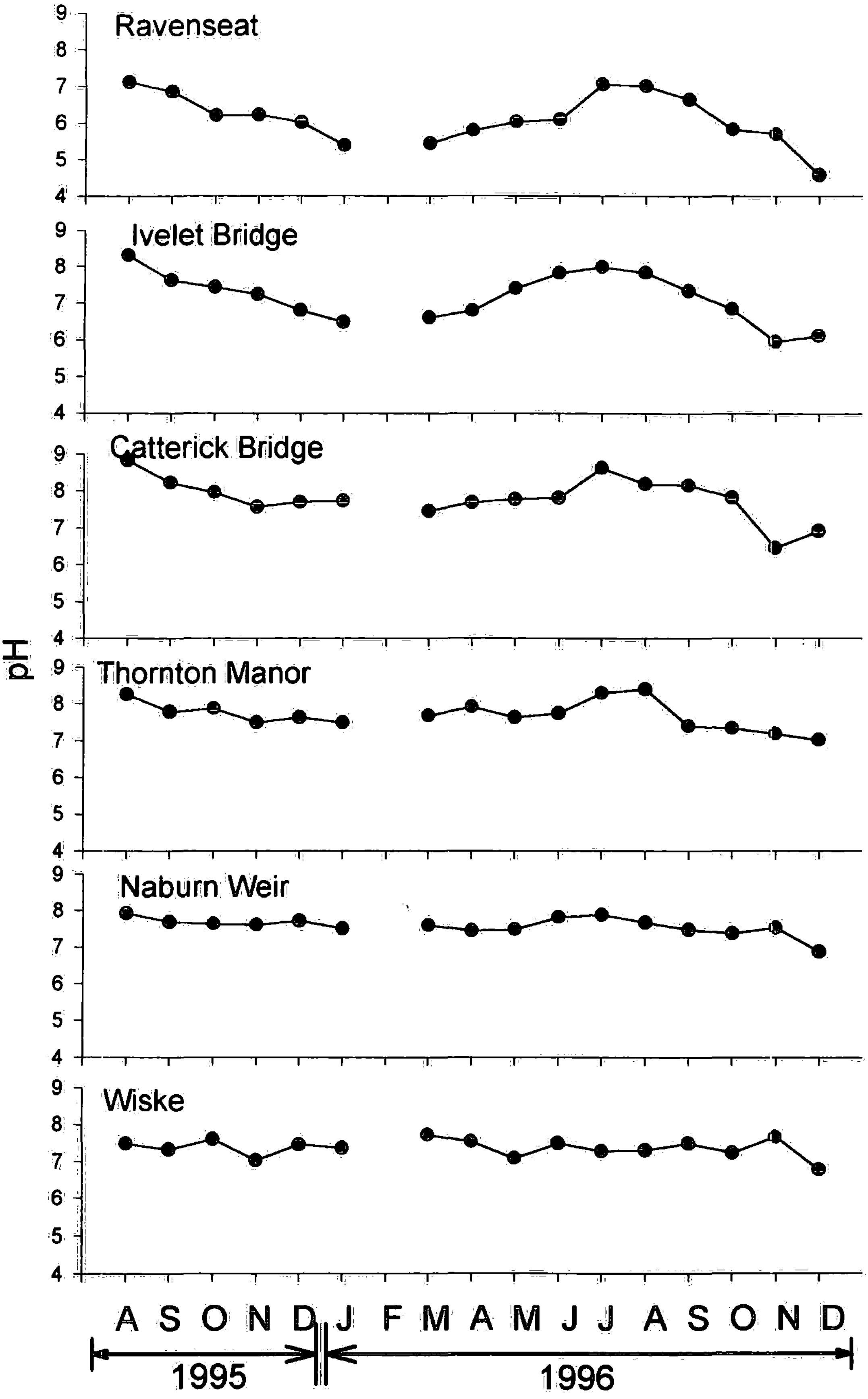


Figure 4.2 Values of pH within the Swale-Ouse system (August 1995 to December 1996)

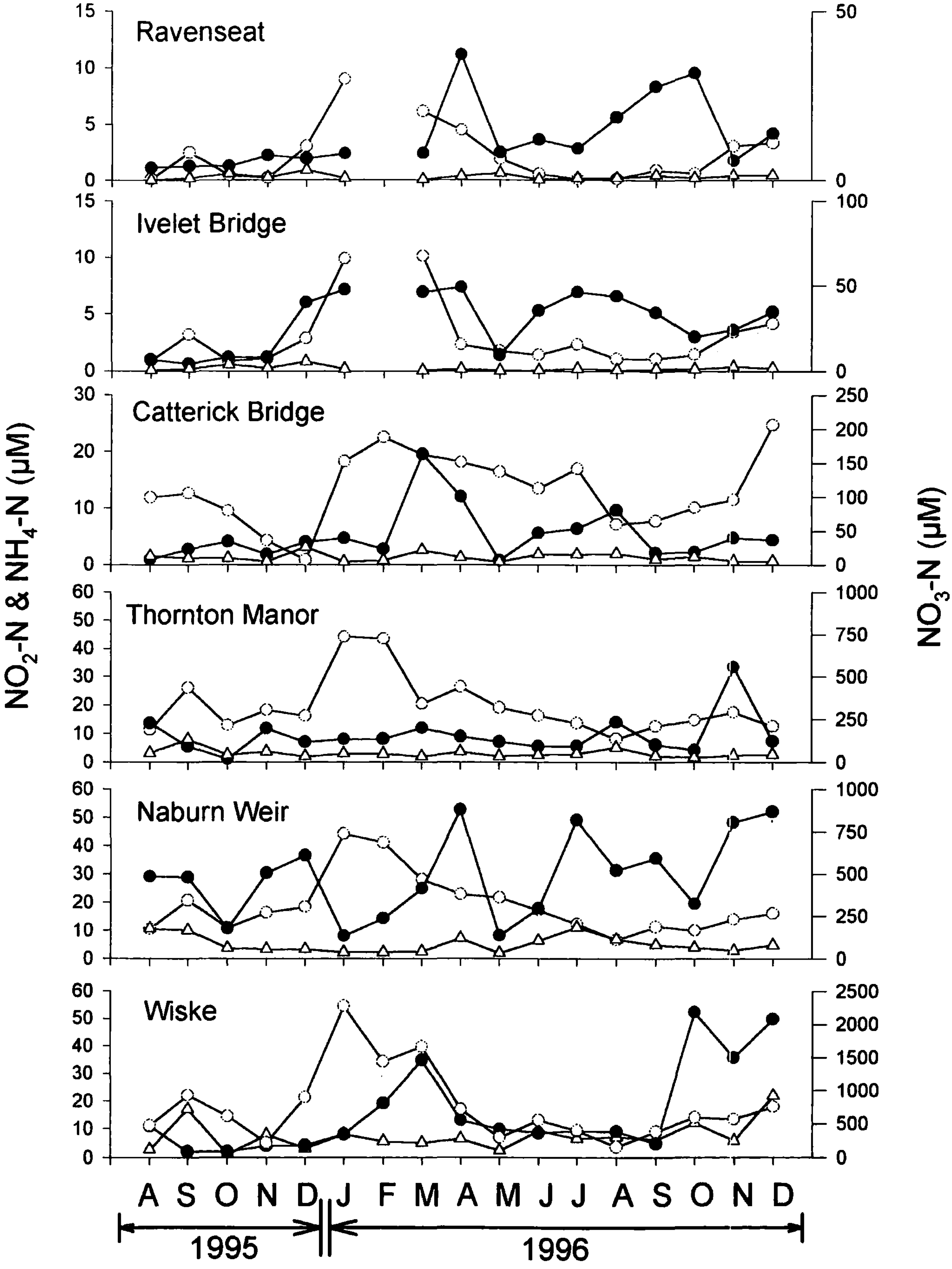


Figure 4.3 NO$_3$-N (open circle), NO$_2$-N (triangle) and NH$_4$-N (full circle) water concentrations obtained monthly from August 1995 to December 1996. Note the different scales between sites and variables.

36 μM NO_3-N, once downstream of Richmond (the first main centre of population) levels in the range of 250 to 750 μM were frequently recorded at Catterick Bridge, Thornton Manor (km 107.9) and Naburn Weir. The Wiske had maximum concentrations on every sample date. Nitrate showed a very clear seasonal trend at all sites, with the highest values being measured during the winter and early spring. For example, on the Wiske, a maximum of 2276 μM NO_3-N was measured in January 1996.

Nitrite and ammonium showed no clear seasonal trends (Figure 4.3); however, spatially they did increase in concentration, again on moving downstream. At the three most upstream sites, nitrite was negligible throughout the sampling programme, often close to detection limits and never exceeding 1.0 μM NO_2-N at Ravenseat and Ivelet Bridge and rarely exceeding 1.5 μM NO_2-N at Catterick Bridge. The highest values for nitrite were usually measured on the Wiske; a maximum of 22 μM NO_2-N was recorded in December 1996. Similarly the highest ammonium values were mainly recorded on the Wiske and Naburn Weir, although values recorded at the upstream sites were often relatively high when compared to the lowland sites.

Phosphorus fractions

Phosphorus concentrations at Ravenseat were relatively low; both organic and inorganic dissolved forms being undetectable at certain times of the year (Table 4.2). At Ivelet Bridge phosphorus concentrations were frequently lower than at Ravenseat due to dilution effects, however, once downstream of Richmond a similar increasing trend to the nitrogen fractions was evident due to anthropogenic inputs. Once again the highest concentrations were measured on the Wiske.

4.23 Sediment variables

Clear seasonal trends for all sites were not so evident for the sediment variables. However, generally total nitrogen and carbon (taken to be an indication of reducible organic substances) in particles less than 100 μm were low in winter and increased in summer (July) and autumn (October) months, for example at Catterick and on the Wiske (Table 4.3).

The sediment data showed a greater trend spatially. The greatest proportion of coarse material (>600 μm) was found at the upstream sites (mean of 43 % at Ravenseat). The percentage silt (<100 μm) gradually increased downstream from

Table 4.3 Sediment variables (means and ranges), measured from January to December 1996 in the Swale-Ouse system.

Ravenseat

	Jan	Apr	Jul	Oct	Mean	1996 Mean	min.	max.
% Particle Size (µm)								
<100	6.1	14.9	8.0	4.2	**8.3**			
100-150	0.9	2.0	0.8	0.9	**1.1**			
150-250	6.5	21.7	8.0	7.7	**11.0**			
250-600	31.0	44.6	27.2	41.5	**36.1**			
>600	55.5	16.9	56.0	45.7	**43.5**			
% Element								
%C	0.200	0.200	0.310	0.130	**0.210**	**0.174**	0.064	0.309
%N	0.010	0.009	0.019	0.009	**0.012**	**0.009**	0.003	0.019
C:N	19.89	21.50	16.36	13.77	**17.88**	**19.50**	13.77	24.35

Ivelet Bridge

	Jan	Apr	Jul	Oct	Mean	1996 Mean	min.	max.
% Particle Size (µm)								
<100	11.0	12.8	9.3	11.9	**11.2**			
100-150	2.1	0.8	0.5	2.6	**1.5**			
150-250	15.3	24.7	7.6	19.8	**16.9**			
250-600	55.1	54.3	37.9	55.0	**50.6**			
>600	16.5	7.5	44.7	10.7	**19.9**			
% Element								
%C	0.280	0.490	0.420	0.300	**0.373**	**0.435**	0.208	0.803
%N	0.013	0.045	0.023	0.017	**0.025**	**0.037**	0.011	0.089
C:N	21.24	10.94	17.73	17.62	**16.88**	**14.83**	2.71	21.24

Catterick Bridge

	Jan	Apr	Jul	Oct	Mean	1996 Mean	min.	max.
% Particle Size (µm)								
<100	28.0	54.0	2.1	9.6	**23.4**			
100-150	2.9	4.0	0.1	2.5	**2.4**			
150-250	25.3	32.2	7.1	12.9	**19.4**			
250-600	37.3	9.3	65.0	53.3	**41.3**			
>600	6.4	0.5	25.7	21.6	**13.6**			
% Element								
%C	0.440	0.377	0.620	1.010	**0.612**	**0.559**	0.205	1.020
%N	0.022	0.020	0.038	0.065	**0.036**	**0.034**	0.011	0.071
C:N	19.89	18.50	16.25	15.56	**17.55**	**17.39**	14.46	19.89

Thornton Manor

	Jan	Apr	Jul	Oct	Mean	1996 Mean	min.	max.
% Particle Size (µm)								
<100	50.8	10.5	26.8	38.8	**31.7**			
100-150	5.5	1.4	4.3	5.2	**4.1**			
150-250	27.3	25.4	32.3	25.5	**27.6**			
250-600	16.1	60.3	35.5	28.3	**35.0**			
>600	0.3	2.5	1.2	2.2	**1.6**			
% Element								
%C	0.370	0.240	0.110	0.260	**0.245**	**0.262**	0.091	0.513
%N	0.028	0.011	0.006	0.023	**0.017**	**0.017**	0.004	0.034
C:N	13.26	21.17	18.95	10.97	**16.09**	**17.61**	10.98	24.08

Naburn Weir

	Jan	Apr	Jul	Oct	Mean	1996 Mean	min.	max.
% Particle Size (µm)								
<100	35.2	28.0	42.2	47.0	**38.1**			
100-150	4.2	7.9	5.2	9.1	**6.6**			
150-250	48.5	48.7	41.0	36.1	**43.6**			
250-600	11.8	14.7	11.1	7.1	**11.2**			
>600	0.2	0.8	0.5	0.8	**0.6**			
% Element								
%C	0.960	0.290	0.430	0.420	**0.525**	**0.517**	0.227	0.958
%N	0.069	0.013	0.024	0.029	**0.034**	**0.034**	0.012	0.069
C:N	13.89	23.03	18.18	14.25	**17.34**	**16.79**	11.39	23.03

Wiske

	Jan	Apr	Jul	Oct	Mean	1996 Mean	min.	max.
% Particle Size (µm)								
<100	9.4	9.2	10.6	10.0	**9.8**			
100-150	2.1	3.2	1.2	0.4	**1.7**			
150-250	16.5	21.5	12.6	12.2	**15.7**			
250-600	63.7	56.0	57.0	58.0	**58.7**			
>600	8.3	10.1	18.6	19.4	**14.1**			
% Element								
%C	0.280	0.210	0.470	0.570	**0.383**	**0.390**	0.214	0.748
%N	0.023	0.016	0.039	0.044	**0.031**	**0.031**	0.016	0.065
C:N	12.18	13.47	12.37	13.22	**12.81**	**12.53**	11.51	13.57

8.3 % at Ravenseat to 38.1 % at Naburn Weir (tidal limit). Total carbon was mainly lower upstream (mean of 0.174 % C at Ravenseat) than downstream (mean of 0.517 % C at Naburn Weir).

Nitrate and ammonium profiles

The profiles of extracted nitrate from sediments at each site from January to December 1996 mirror the trends in nitrate concentration evident within the water column (Figs. 4.4 & 4.5). The concentration of nitrate in the sediment increases again on moving from upland to lowland sites. For example in November 1996 the concentrations of nitrate measured in the upper centimetre at Ravenseat, Naburn Weir and the Wiske were 7 $\mu g\ N\ g^{-1}$, 13 $\mu g\ N\ g^{-1}$ and 18 $\mu g\ N\ g^{-1}$ by dry weight of sediment respectively. Again the highest concentrations were always extracted from Wiske sediment. Additionally, similarly to the water column, the concentration of nitrate in the sediment varies seasonally, a summer minimum and a winter maximum is evident for all sites. A trend is also depicted through the sediment profiles, generally the concentrations of nitrate were higher in the upper centimetres than in the deeper sediment, particularly in the winter months, for example in November and December at Catterick Bridge (Fig. 4.4).

The profiles for extracted ammonium (Figs. 4.6 & 4.7) again show a tendency to increase in concentration moving from upland to lowland sites. The highest concentrations were extracted from Wiske sediment, a maximum of 50 $\mu g\ N\ g^{-1}$ dry wt. being recorded for this site. Similarly to ammonium concentration in the water column, there is not as strong a seasonal trend as with the nitrate data. A trend that is more apparent in the sediment cores is for an increase in concentration with depth, particularly for Catterick Bridge (Fig. 4.6), Thornton Manor and the Wiske (Fig.4.7).

Microbial numbers

Most Probable Number (MPN) counts for denitrifiers generally increased with distance from source in sediments collected in October 1996 (Table 4.4).

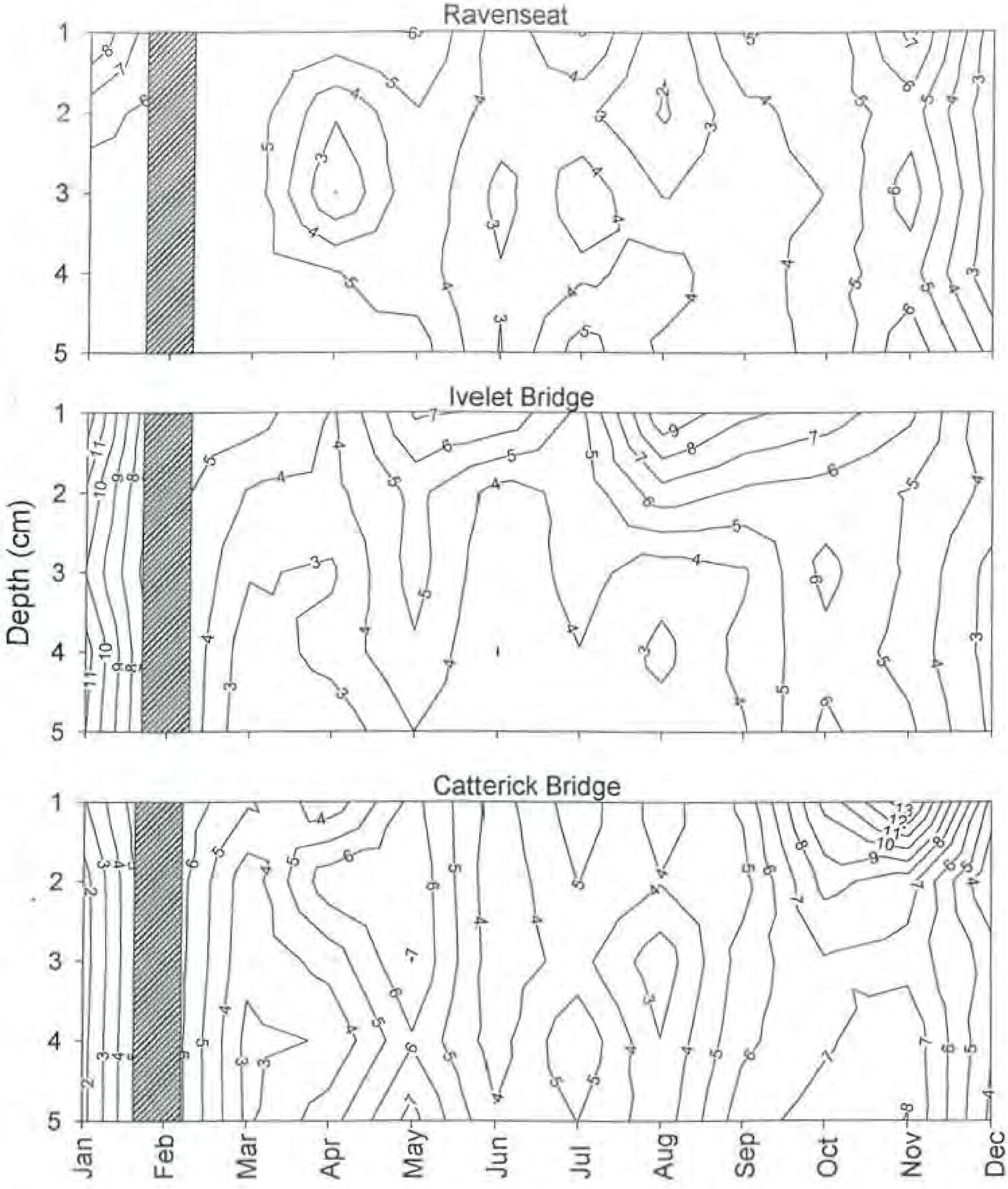


Fig. 4.4 Profiles of extracted nitrate in Swale-Ouse sediments (NO_3-N µg g^{-1} d.wt.)
Measured from January to December 1996 (shaded bar indicates missing data)

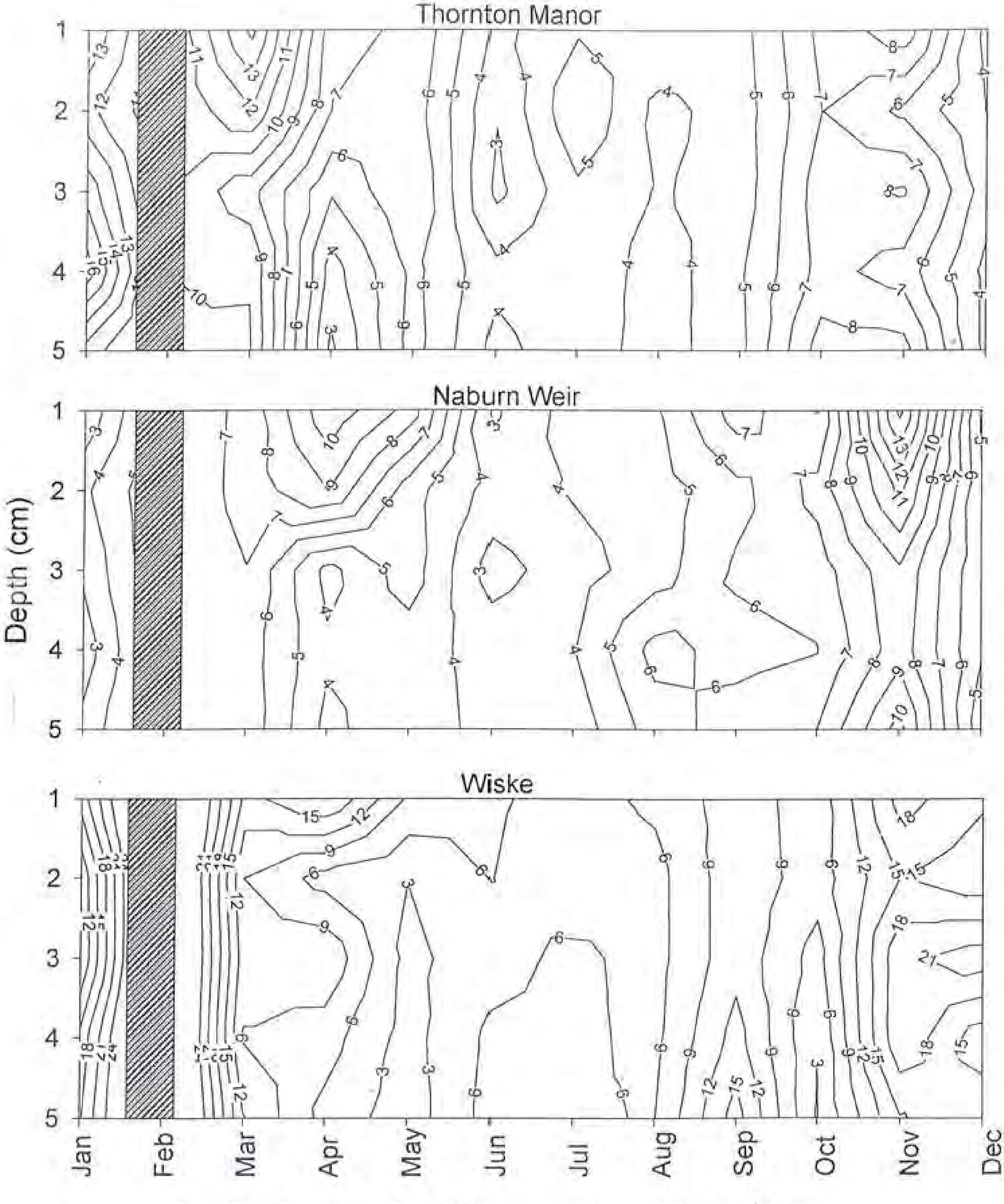


Fig. 4.5 Profiles of extracted nitrate in Swale-Ouse sediments (NO_3-N µg g^{-1} d.wt.) Measured from January to December 1996 (shaded bar indicates missing data)

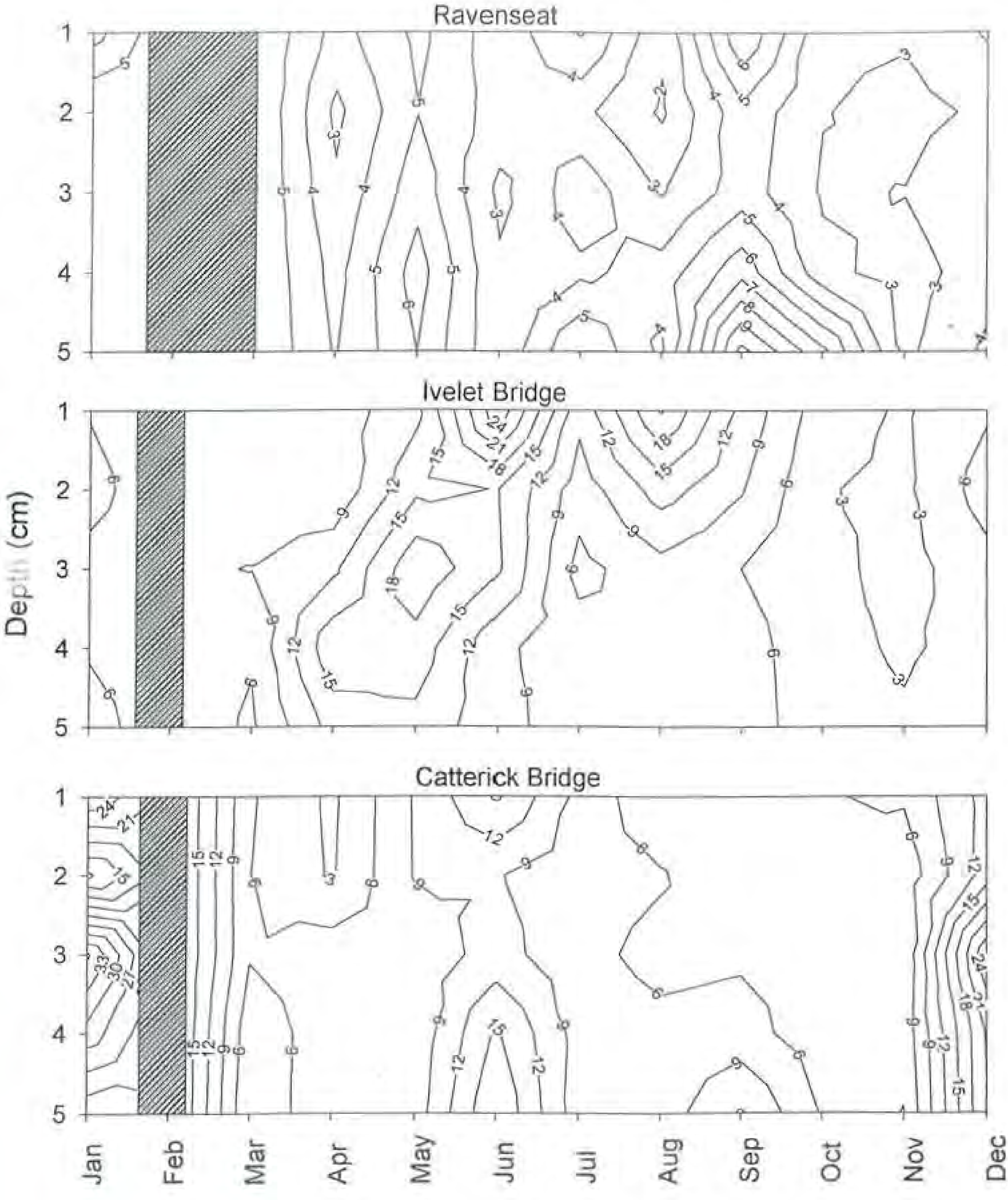


Fig. 4.6 Profiles of extracted ammonium in Swale-Ouse sediments (NH$_4$-N μg g^{-1} d.wt.) Measured from January to December 1996 (shaded bar indicates missing data)

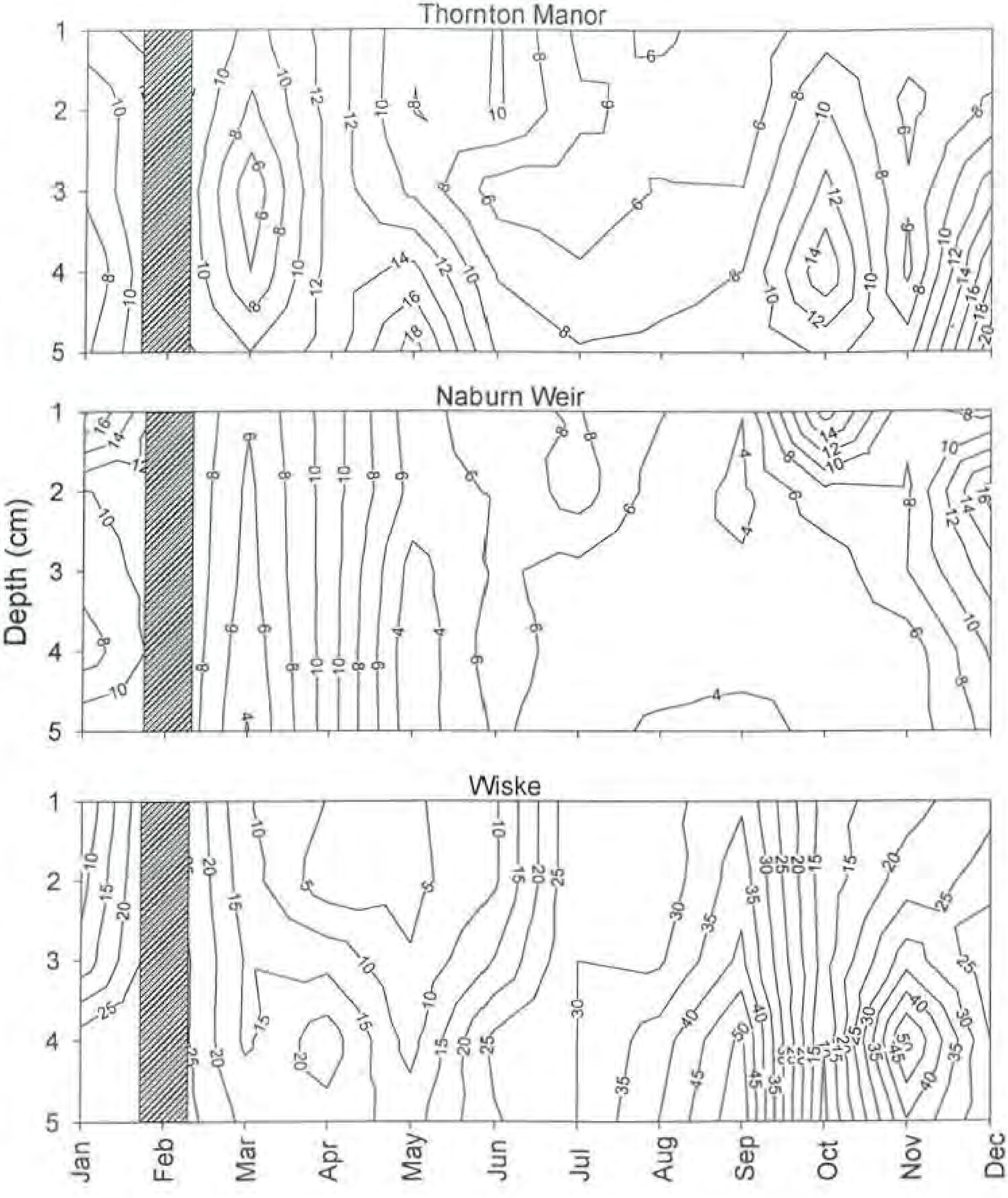


Fig. 4.7 Profiles of extracted ammonium in Swale-Ouse sediments (NH_4-N μg g^{-1} d.wt.) Measured from January to December 1996 (shaded bar indicates missing data)

Table 4.4 Most Probable Number estimates for denitrifiers in Swale-Ouse sediments (16/10/96)

Site	**MPN (g^{-1} dry weight sediment)**
Ravenseat	24
Ivelet Bridge	9885
Catterick Bridge	2200
Thornton Manor	1400
Naburn Weir	90523
Wiske	27375

4.3 Sediment denitrification

For comparison with the above environmentally controlling factors, the variation between sites and seasons in denitrification rates, measured using intact sediment cores, along the Swale-Ouse continuum is shown in Figure 4.8.

Spatial trend

A consistent increase (up to 30 times) in sediment denitrification rate on moving downstream was found (Fig. 4.8). The lowest rates (rarely exceeding 20 μmol N m^{-2} h^{-1}) were found at the two upstream sites, Ravenseat and Ivelet Bridge. Similarly to nitrate concentrations, the rates showed a gradual increase downstream, the highest rates (883 ±134 μmol N m^{-2} h^{-1}) were recorded on the Wiske.

Seasonal trend

A seasonal trend in denitrification is also evident from Figure 4.8. The lowland sites all showed a peak in activity in the spring months (March to May), for example at Catterick Bridge a rate of 279±56 μmol N m^{-2} h^{-1} was recorded in April 1996 compared to 48.95±9.81 μmol N m^{-2} h^{-1} the previous January. During the summer months there was a general decrease in activity, rates at Catterick had dropped to 87±21 μmol N m^{-2} h^{-1} by August, through to the winter months. Similarly, Naburn Weir had a peak denitrification rate of 659±187 μmol N m^{-2} h^{-1} in May 1996, dropping to 40±15 μmol N m^{-2} h^{-1} by July 1996, a winter low of 21±15 μmol N m^{-2} h^{-1} was recorded in December 1995. The two upland sites showed no marked seasonality.

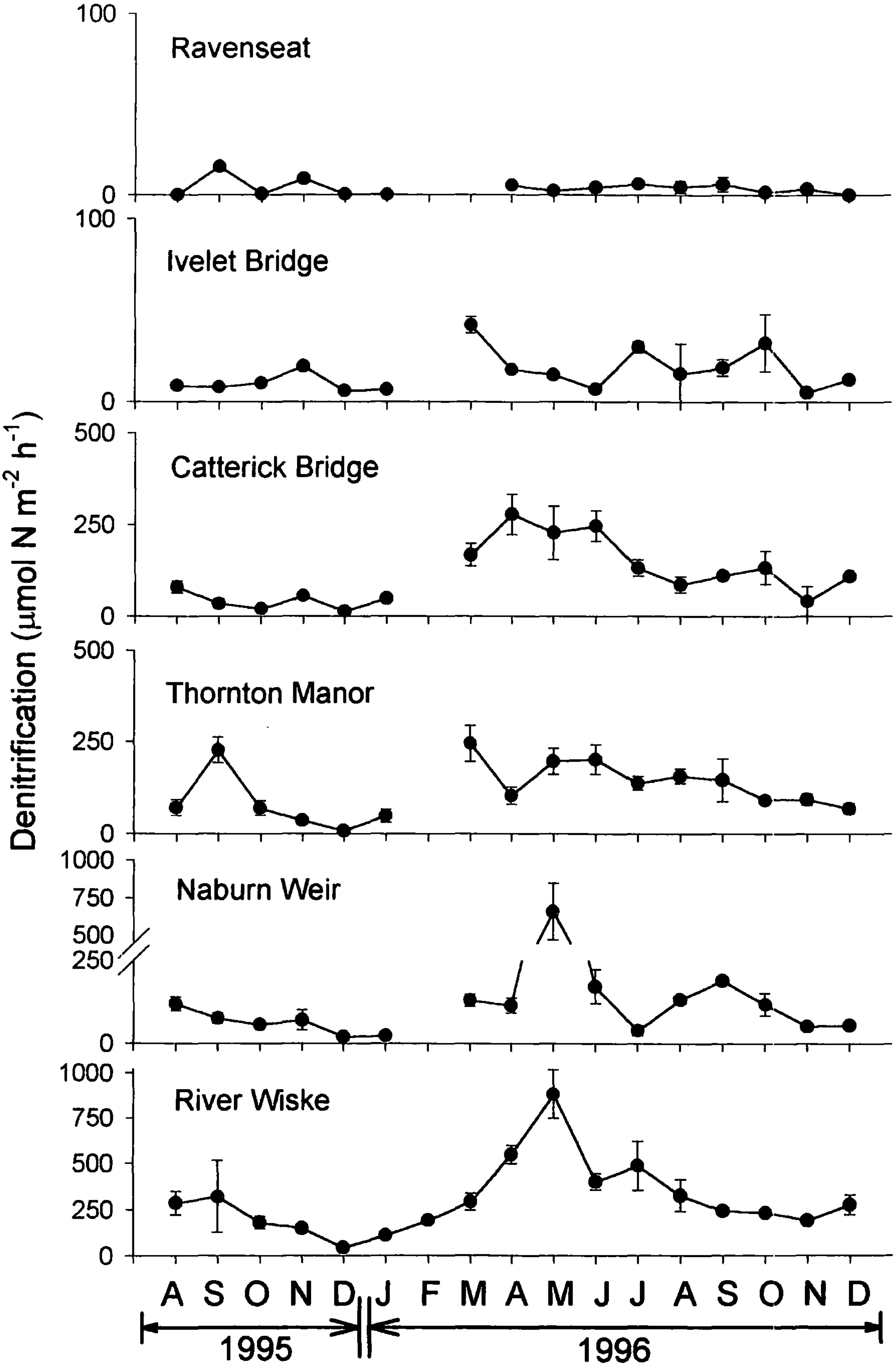


Figure 4.8 Denitrification rates in intact sediment cores obtained monthly from August 1995 to December 1996. Bars denote the standard error of three replicates. Note the difference in scales between sites.

Principal Components Analysis

In order to assess and illustrate the seasonal and spatial trends evident within the data set for the Swale-Ouse system from January to December 1996, a principal components analysis (PCA) was performed. Before analysis, the data (except for pH) were normalised using log transformation.

This analysis served to place each attribute on constructed axes (components) so that its geometrical position relative to other attributes reflected similarities between them. Only the first two components were considered as they described 79 % of the data between them. The first component represented 64 % and the second described 14 %. It is evident from the correlations of the environmental variables with the first two components that the first axis is analogous to the spatial variation in these variables (Fig. 4.9). The nutrient fractions, denitrification and pH are all strongly negatively correlated with Principal Component 1 (PC 1), suggesting that as this axis becomes more negative, so the distance from source is greater. All of these variables increase with distance from source. PC 2 describes much less of the data, however, the positioning of the environmental variables along it might suggest that it represents a seasonal trend within the data set. pH is strongly negatively correlated with this axis and, as previously discussed, exhibits seasonality particularly at the upstream sites (Fig. 4.2) where it decreases within winter months. The nutrient fractions are more positively correlated with this axis, nitrate in particular exhibits strong seasonality, increasing in the winter months (Fig. 4.3). The positioning of denitrification on this axis falls between pH and nitrate, this exhibits a peak in activity in the spring (Fig. 4.8). Thus, the relationship of these particular variables to PC 2 might suggest that the negative end of the scale represents summer months and the positive end, winter months.

Plotting the individual samples using their component scores reinforces the interpretation that PC 1 represents a spatial trend and PC 2 represents a seasonal trend (Fig. 4.10). The samples are orientated along the first component ranging from the most upstream site, Ravenseat, down to Naburn Weir, the most downstream site on the main river. The Wiske is located at the most negative end of the scale, as previously shown it generally exhibited the highest nutrient concentrations, conductivity and denitrification activity. The second component would appear to separate winter months from summer months. The winter samples are generally located at the more positive end of the site groupings, whereas the summer samples are at the more negative end of the scale. The strong influence of pH on this axis could lead to the greater spread of

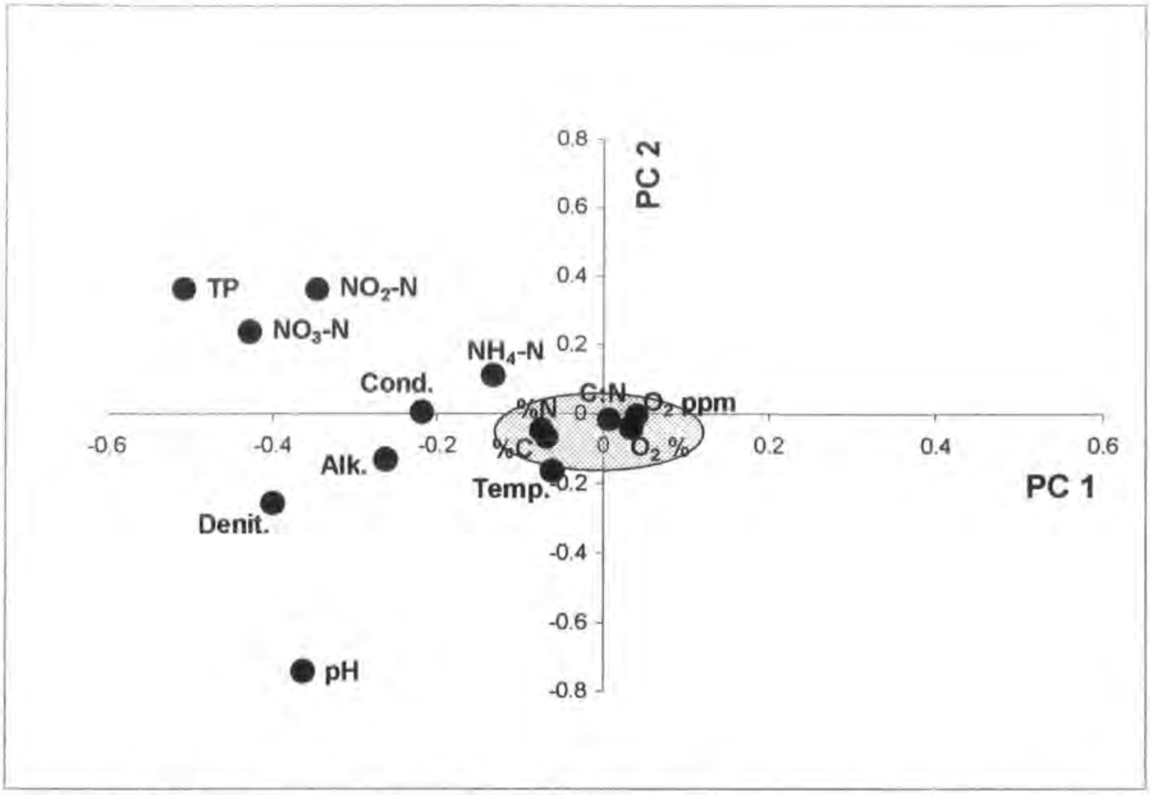


Figure 4.9 Principal components analysis of Swale-Ouse data. Correlations of the environmental variables with the first two components. Shaded area indicates below 95 % confidence levels. TP = Total Phosphate; Cond. = Conductivity; Denit. = Denitrification; Alk. = Total Alkalinity; Temp. = Temperature.

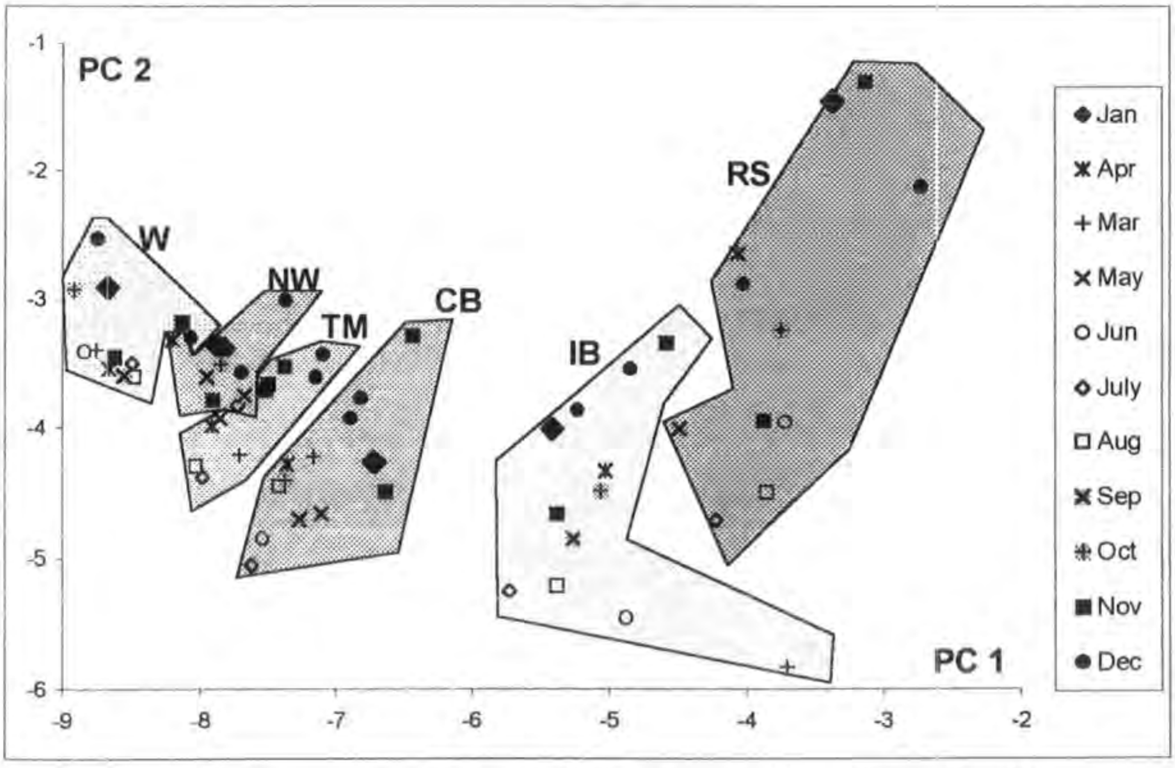


Figure 4.10 Principal components analysis of Swale-Ouse data. Position of the samples with respect to the first two components.

samples at the more upstream sites where pH is more variable seasonally than further downstream.

In analysing these data using PCA multivariate analysis, it must be noted that this approach is inductive and non-experimental in order to highlight patterns in the data, as opposed to the deductive approach of most other statistical analysis which seek a mathematical relationship between variables.

Correlation

In an attempt to deduce the relative influence of the measured *in situ* environmental variables on denitrification activity for each site through the year, correlation analysis was performed on the data, normalised by log transformation. No significant correlations ($p<0.05$) between denitrification and any of the *in situ* environmental variables were found at any site. However, when these data for all sites were pooled to investigate effectively the relationships between variables from upstream to downstream, as opposed to seasonal relationships which the single site correlation assessed, significant relationships ($p<0.001$) were found (Table 4.5).

Table 4.5 Pearson correlation matrix of all Swale-Ouse log-transformed data (August 1995 to December 1996) Shading indicates $p<0.001$. Denit = Denitrification; Cond = Conductivity; TP = Total Phosphate; Temp = Temperature; Alk = Alkalinity.

	Denit	**Cond**	**%C**	**NO_3-N**	**NO_2-N**	**NH_4-N**	**TP**	**pH**	**Temp**	**%O_2**	**Alk**	**%N**
Cond	0.822											
%C	0.422	0.360										
NO_3-N	0.760	0.832	0.409									
NO_2-N	0.618	0.751	NS	0.738								
NH_4-N	0.424	0.482	NS	0.524	0.568							
TP	0.716	0.850	0.306	0.813	0.884	0.476						
pH	0.606	0.597	0.384	0.429	0.340	NS	0.419					
Temp	0.305	0.283	NS	NS	NS	NS	0.271	0.501				
%O_2	-0.376	-0.599	NS	-0.405	-0.478	NS	-0.519	NS	NS			
Alk	0.756	0.762	0.427	0.665	0.555	0.298	0.654	0.562	0.364	-0.366		
%N	0.401	0.357	0.902	0.395	NS	NS	NS	0.296	NS	NS	0.371	
C:N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.594

Particularly strong correlations were found between denitrification and nitrate, nitrite, conductivity, phosphate, pH and alkalinity (Table 4.5). This highlights the fact that these data exhibit a high degree of collinearity. Conductivity, nitrate, nitrite and phosphate are closely related ($p<0.001$) as they all increase with greater anthropogenic input downstream. Additionally, as previously discussed, pH, alkalinity and conductivity are all interrelated, hence the strong correlations ($p<0.001$). Temperature is not so significantly correlated because this correlation assesses the changes from upstream to downstream as opposed to seasonal effects.

Regression

A best subsets regression of all water and sediment variables analysed in the 17 month survey was used to assess which variables exerted the greatest combined influence on denitrification in the Swale-Ouse. Taking into account the strong collinearity evident within this data set, water nitrate and temperature explained 68 % of the total variance in the data. Multiple regression analysis was then applied to these field data to assess the relationship between denitrification and the controlling variables nitrate and temperature. Equation 1 describes the relationship of the log-transformed variables.

$$\text{Denitrification } (\mu\text{mol N m}^{-2}\text{ h}^{-1}) = -1.41 + 0.738\ \text{NO}_3\text{-N}(\mu\text{g L}^{-1}) + 0.841\ \text{Temp}(^\circ\text{C}) \quad (1)$$

4.31 Spatial heterogeneity in sediment denitrification

Two sets of three replicate cores were taken from different positions in a 10 m reach on the Wiske at Castle Farm on 2 April 1996, and denitrification rate and N_2O production determined. The positions were chosen to represent each of the following: A) coarse sand; B) sand and gravel; C) fine sand over compacted clay; D, E, F) fine sand and silt covered with plant debris. The cores were incubated for 4 h at 15°C in the dark.

The results of this heterogeneity study are summarised in Table 4.6. Comparison of different types of substrate at one site showed a four-fold difference between the lowest value of 99 μmol N m^{-2} h^{-1} in fine sand over compacted clay to 382 μmol N m^{-2} h^{-1} in the fine sand plus silt covered by plant debris.

Table 4.6 Denitrification rate and N_2O production in different types of sediment in the Wiske (km -1.7, 86.1) on 4 April 1996 (mean ± SD; n = 3).

Site	Characteristics of the sediment	Denitrification rate (μmol N m^{-2} h^{-1})	N_2O production (μmol N_2O-N m^{-2} h^{-1})
A	Coarse sand	140 ± 28.1	24.7 ± 21
B	Sand and gravel	134 ± 60.5	-6 ± 8.3
C	Fine sand over compacted clay	99 ± 5.04	113.6 ± 27
D	Fine sand and silt with plant debris	360 ± 23.7	27.7 ± 54.6
E	Fine sand and silt with plant debris	261 ± 107	3.8 ± 24.3
F	Fine sand and silt with plant debris	382 ± 51.0	30.4 ± 29.3

4.4 Nitrous oxide (N_2O) production

The study showed that changes in N_2O production (intact sediment cores without acetylene) during the assays ranged from a slight net uptake to marked release (Table 4.7). Low or slightly negative values were sometimes recorded at all sites, but there was a marked tendency for maximum values to increase on passing downstream. For example, at Ivelet Bridge, the values ranged from -18.5 μmol N_2O-N m^{-2} h^{-1} (i.e. net uptake) to 14.0 μmol N_2O-N m^{-2} h^{-1} (i.e. net release). Downstream at Naburn Weir values ranged from 1.4 to 100 μmol N_2O-N m^{-2} h^{-1} and for the Wiske, values ranged from 0.13 to 497 μmol N_2O-N m^{-2} h^{-1}.

Table 4.7 N_2O production in the sediments in the Swale-Ouse and Wiske rivers during 1996. Samples were collected monthly, apart from February for the main river sites.

River	Site	Distance (km)	N_2O production (μmol N_2O-N m^{-2} h^{-1})		
			min	mean	max
Swale					
	Ravenseat	-2.5, 0.0	-1.2	0.8	2.8
	Ivelet Bridge	10.9	-18.5	0.1	14.0
	Catterick Bridge	49.9	-0.4	43.8	96.4
	Thornton Manor	107.9	3.1	32.5	65.7
	Naburn Weir	145	1.4	30.8	100.0
Wiske					
	Wiske	-1.7, 86.1	0.1	161.0	497.0

No clear seasonal trend was evident at Ravenseat or Ivelet Bridge, but the downstream sites showed some tendency for seasonal changes (Figs. 4.11 and 4.12). A winter low is evident for all sites, however, values tended to be lower in the latter part

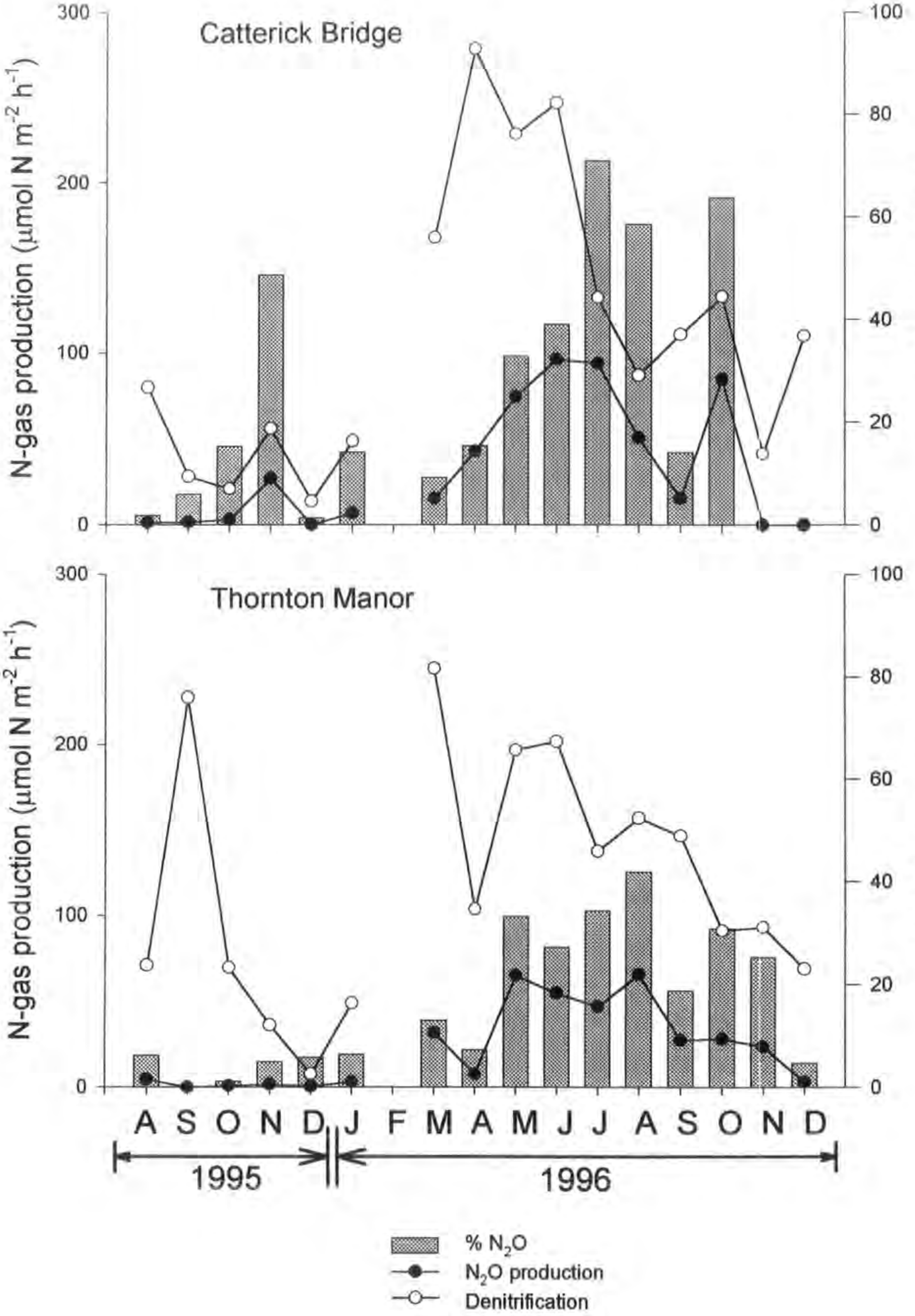


Figure 4.11 Nitrous oxide production from sediments at Catterick Bridge and Thornton Manor from August 1995 to December1996.

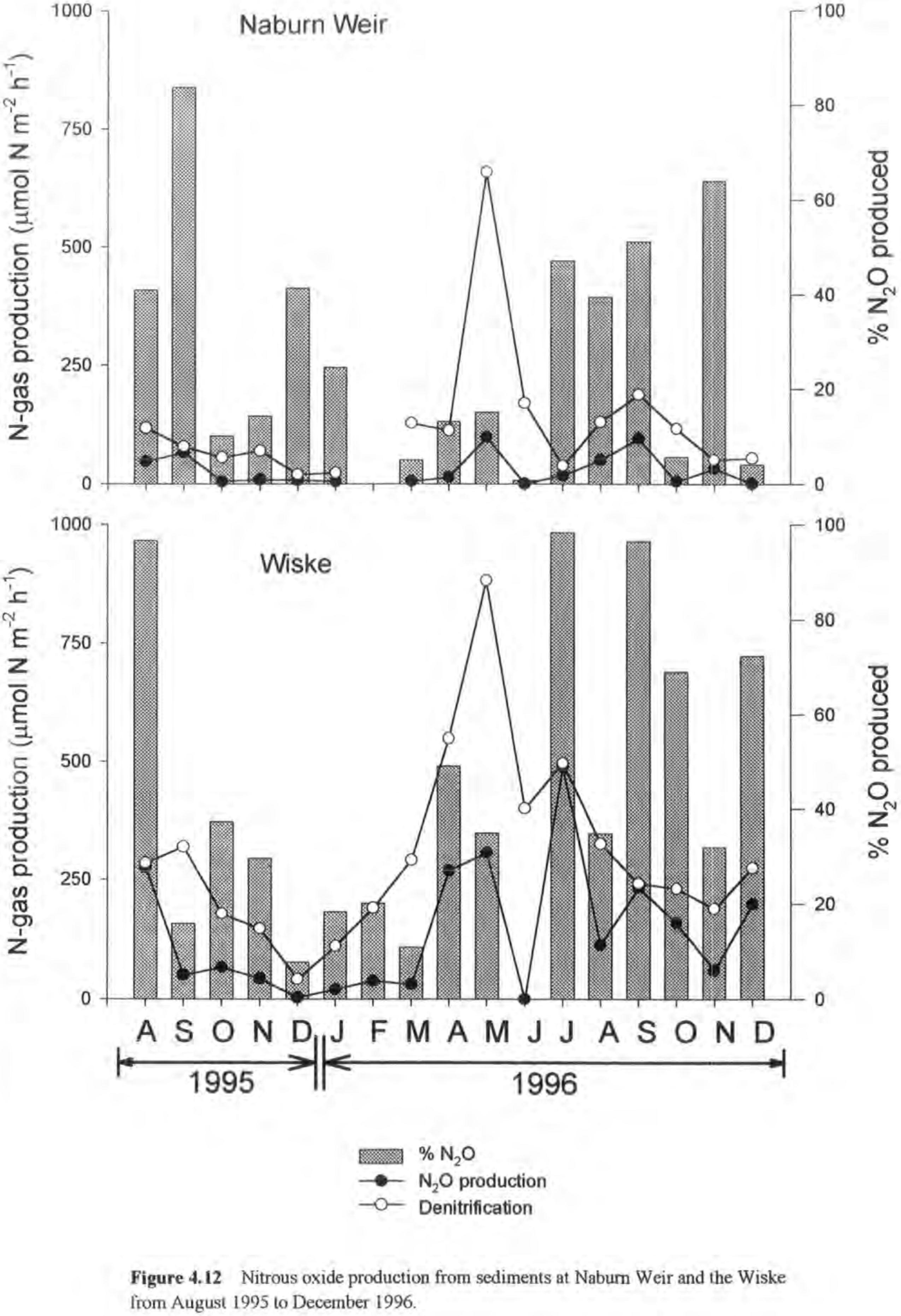


Figure 4.12 Nitrous oxide production from sediments at Naburn Weir and the Wiske from August 1995 to December 1996.

of 1995 than in the same months of 1996. This effect was even more clear for the Wiske. There is an increase in N_2O production in spring, this is particularly apparent at Catterick Bridge (Fig. 4.11) and the Wiske (Fig. 4.12), peaking in the summer months. The highest production rate of N_2O at 497±184 μmol N_2O-N m^{-2} h^{-1} was measured on the Wiske in July 1996.

The proportion of N_2O release related to the total gases released during denitrification ($N_2O + N_2$ from intact sediment cores with acetylene) showed great variation (minimum 0, maximum 100 %) as indicated by the bars on Figs. 4.11 and 4.12. Again there was a general seasonal trend towards higher proportions within the summer months compared to the winter months. The greatest proportions of N_2O were released from Wiske sediments, a maximum of 100 % was measured in July 1996.

The variability (coefficient of variation, CV) of measurements for N_2O production at a particular site on any one day tended to decrease on passing downstream (Table 4.8). Data on variability in denitrification rate at the same time are included in this table; these show that the annual mean for the CV of N_2O production was more than twice that for denitrification rate at all the sites.

Table 4.8 Comparison of variability of results for N_2O production and denitrification during 1996, as shown by the mean annual values for the coefficients of variation of the two processes.

River	Site	Distance (km)	Coefficient of variation	
			N_2O production (μmol N_2O-N m^{-2} h^{-1})	Denitrification (μmol N m^{-2} h^{-1})
Swale				
	Ravenseat	-2.5, 0.0	116	37.5
	Ivelet Bridge	10.9	61.2	22.2
	Catterick Bridge	49.9	109.5	24.4
	Thornton Manor	107.9	89.9	21.6
	Naburn Weir	145.0	49.0	22.2
Wiske				
	Wiske	-1.7, 86.1	35.7	17.0

In an attempt to assess the relative influence of the environmental variables (temperature, NO_3-N, NH_4-N, PO_4-P) and denitrification on N_2O production, correlation analysis was performed on the data (normalised by logarithmic transformation) for each site through the year. There was no significant correlation (p <0 .05) between N_2O production and any environmental variable. However, when the relationship between N_2O production and environmental variables was assessed for the data from all six sites on any one day, there was a significant positive correlation ($p < 0.05$) with NO_3-N concentration on 7 of the 11 days during 1996 (11 of 16 during whole study) (Table 4.9). The annual mean value for N_2O production at any one site also showed a significant positive relationship ($p < 0.05$) with the annual mean value for NO_3-N concentration at the same site (Fig. 4.13).

Table 4.9 Relationship between N_2O production and nitrate in the water column at particular dates for all the sites. R^2 indicates the coefficient of determination for the curves. Slope indicates the increase in N_2O production (μmol N_2O-N m^{-2} h^{-1}) for each μM nitrate increase in the water column.

Sampling date	R^2	Slope
1995		
16 August	0.84	0.58
12 September	0.32	-
17 October	0.80	0.10
14 November	0.01	-
18 December	0.16	-
1996		-
15 January	0.85	7.66
15 March	0.60	-
18 April	0.40	-
16 May	0.01	-
20 June	0.66	0.63
15 July	0.88	0.65
16 August	0.82	1.47
17September	0.70	0.24
15 October	0.92	8.41
22 November	0.73	0.10
12 December	0.77	0.03

4.5 Tidal reaches

Physical and chemical variables, water temperature, conductivity, oxygen, pH and nitrate were recorded from March to early October 1996, and denitrification rate was determined monthly from July to October within the 21-km freshwater tidal stretch of the Yorkshire Ouse.

Three sites were sampled routinely in this supplementary survey. These included the upstream tidal limit (immediately downstream of Naburn Weir), an intermediate site (Cawood) and the downstream freshwater limit (Selby). These sites were sampled without the use of a boat. The Naburn Weir site was reached from the shore, while the Cawood and Selby sites were sampled from jetties.

Samples and measurements were taken approximately 1 m from the jetty or other position of the sampler and at approximately 10 cm below the surface. One

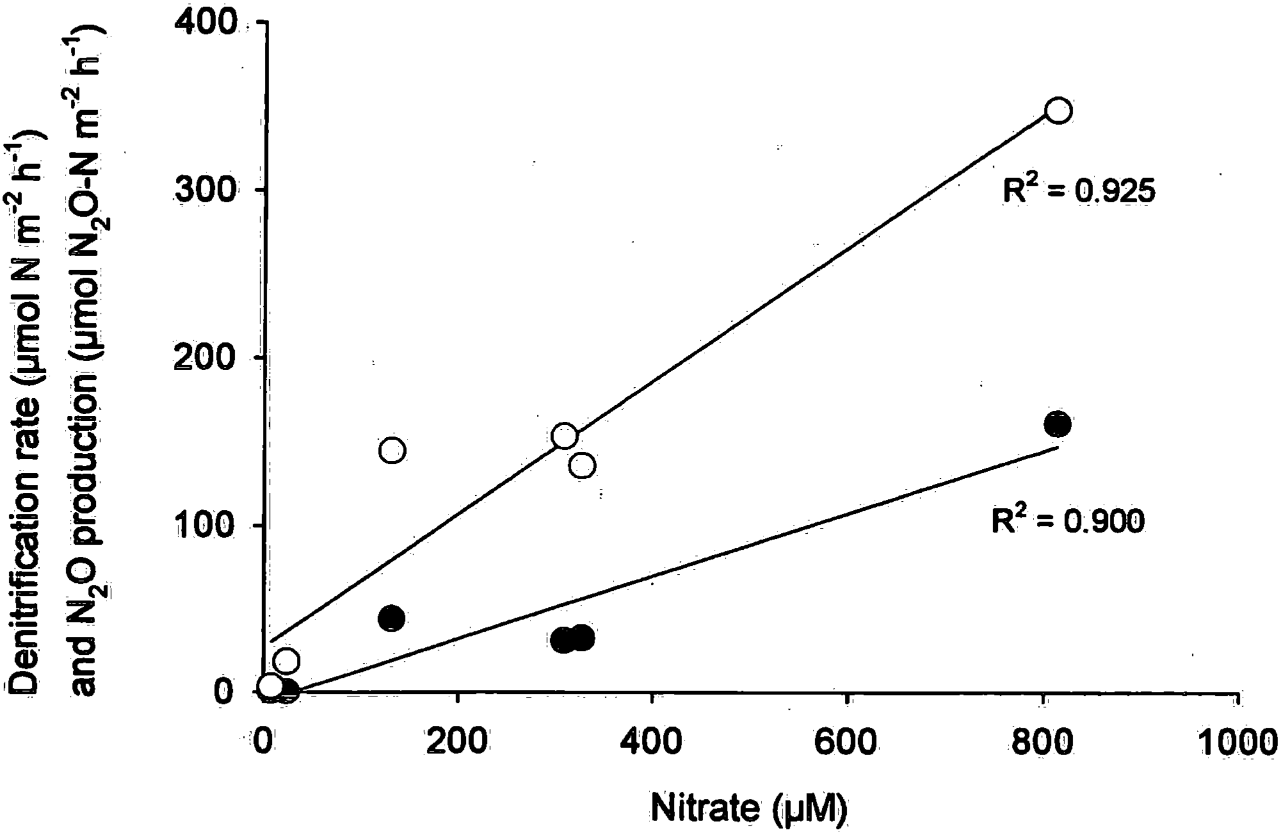


Figure 4.13 Relationship between the annual mean denitrification rate (open circles) and N_2O production compared to annual mean NO_3-N (filled circles) at any one site on the Swale-Ouse system

sample was also taken (Selby, 4 July 1996) to test whether denitrification was detectable in the water column. Sediment cores were taken from positions close to where the water was sampled. The greatest depth of overlying water at the position sampled was 4 m at Selby (although the maximum river depth here is several metres deeper). The cores were collected using a 'Mackereth' sediment corer (Freshwater Biological Association, Cumbria), which includes a 50 cm deep and 5.5 cm internal diameter plastic cylinder. The sediment sample was typically about 10 cm deep, with the rest of the cylinder being filled with water. A sub-sample of this core was taken at the site by means of one of the narrower plastic cylinders (25 cm height, 3.5 cm internal diameter) used for the studies on the non-tidal river. (This was done because of the need to transfer each individual sample of sediment to the laboratory in its own cylinder.) The narrower cylinder was placed vertically over the centre of the broader cylinder and plunged into the core. It proved possible to do this with minimum disturbance to the core; as there was little sign of particles being disturbed from the surface in the narrower cylinders.

The narrower cylinder was sealed with a rubber bung and removed carefully from the broader cylinder. The bottom of the core was sealed with an additional rubber bung. The cores were transported to the laboratory in an ice box. Three triplicate sets of three cores were collected for denitrification and N_2O production assays. An additional set of cores was collected on 1 October 1996 at the three main sample sites to determine the water content, total carbon and nitrogen and particle size composition of the sediment.

Some environmental features of the tidal sites are summarised in Figure 4.14. Temperature increased rapidly through the spring months to peak in August at all sites (Fig. 4.14a). Conductivity remained relatively constant with the highest levels generally recorded at Selby (km 230), similarly, pH remained stable throughout the sampling period (Fig. 4.14b & d). The water column oxygen concentrations were always over 87 % at Naburn Weir, however, an oxygen sag was evident in the summer months downstream at Cawood (km 210) and Selby where concentrations dropped to 43 and 22 % respectively in August (Fig. 4.14c). There was no difference in nitrate concentrations between sites, although concentrations decreased from March through to October when a minimum of 137 µM was found at Cawood (Fig. 4.14e).

The cores were always some shade of brown; even at Selby, there was no evidence of a black anoxic layer. The sediment characteristics measured on one day show (Table 4.10) that the proportion of fine particles (< 100 µm) in the sediment at

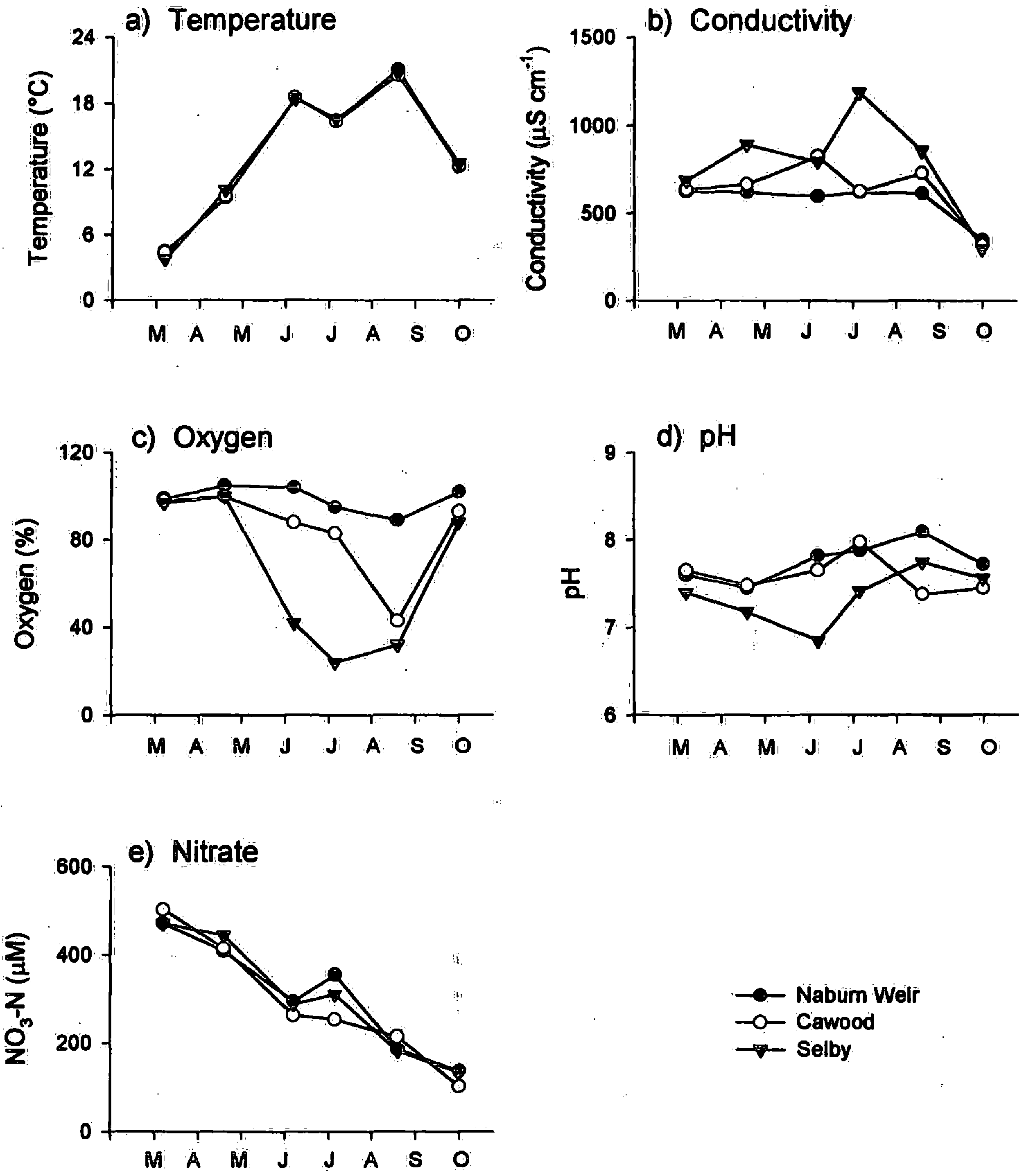


Figure 4.14 Physical and chemical data for the freshwater tidal reaches of the River Ouse (March to October 1996).

Naburn Weir was less than one sixth of that at Cawood or Selby (95.1 %). More than 50 % particles in the sediment were fine sand for Naburn Weir, but only 6.1 and 3.2 % for Cawood and Selby, respectively. The composition of the particles < 100 μm also changed on passing downstream: C and N at Naburn Weir were both more than ten times lower than at Cawood or Selby. There was a progressive increase in the C : N ratio of these particles on passing downstream (19.5 to 48.4: Table 4.10)

Table 4.10 Sediment variables in the freshwater tidal reaches of the Yorkshire Ouse (1/10/96).

Sediment variable	Naburn Weir	Cawood	Selby
% Particle Size (μm)			
< 100	13.7	90.6	95.1
100-250	61.5	6.1	3.2
>250	23.6	2.3	0.5
% Element			
%C	0.12	3.37	2.07
%N	0.006	0.088	0.042
C:N	19.5	38.0	48.4

Sediment denitrification rates ranged from below detection limits in early October at Selby to 378±21 μmol N m^{-2} h^{-1} at Cawood in August (Fig. 4.15). The denitrification rate decreased from June to October 1996 at Naburn Weir, but no trend was apparent at Cawood or Selby. Spatially, generally denitrification decreased from the tidal limits at Naburn Weir to Selby.

Denitrification was not detected in the water column at Selby by either acetylene block or ^{15}N techniques, despite the very low dissolved oxygen concentrations.

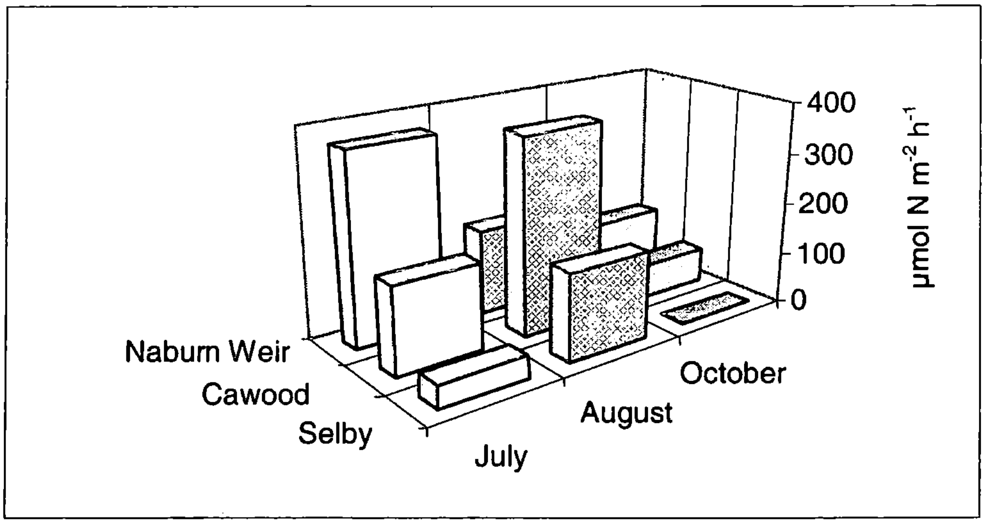


Figure 4.15 Sediment denitrification measured in sediment cores from the freshwater tidal reaches of the River Ouse (July to October 1996).

4.7 Discussion

The results of this chapter show that seasonal and spatial trends are evident in both the environmental and denitrification data along the Swale-Ouse system. This therefore suggests that the hypotheses (Section 1.4), that a gradient of physical and chemical variables will elicit a graded response from denitrifying bacterial populations and that seasonal variations in these variables will also elicit a response, can be accepted.

PCA and statistical analyses (Section 4.3) have helped to illustrate and provide an explanation to these findings, by assessing the spatial and seasonal trends evident within the data and by deducing the relative influence of measured *in situ* environmental variables on denitrification activity. A high degree of colinearity is evident between environmental variables; for example, alkalinity was strongly positively correlated with conductivity (p<0.001) due to the fact that conductivity increases with increased ions in the water column, such ions would include carbonates, thus resulting in the simultaneous increase in alkalinity and hence the buffering capacity of the water. Despite the colinearity within the data set, a significant relationship between denitrification, nitrate and temperature was found through multiple regression (Section 4.3).

Conductivity, alkalinity, pH and nutrient fractions all increased with distance from source, mostly exhibiting a peak on the eutrophic Wiske. This is probably due to the fact that urban and agricultural activities are greatly increased in the lowland reaches. Seasonal fluctuations were observed in flow, temperature, pH and nitrate. Flow and nitrate showed a peak in winter months, probably as a result of increased run-off, whereas temperature and pH (particularly at the upstream sites) peaked in the summer months. Extracted nitrate and ammonium fractions from sediments mirrored the trends of these fractions in the water column, although not to such a great extent, possibly as a result of utilisation by bacterial populations. The proportion of sediment carbon and silt generally increased with distance downstream, again with a maximum measured on the Wiske. This increase in sedimentation is possibly indicative of the surface run-off from the agricultural lowland catchment area, as well as decreases in current velocity; furthermore, this increase has direct consequences on the environmental conditions within these sediments. For example, at Ravenseat there was a low C %, yet a high percentage of sand and gravel, therefore it would be expected that the sediments would be well aerated, in addition there would be little surface area for microbial attachment. Further downstream, the C % increase is mirrored by the percentage of silt, which whilst potentially decreasing the penetration of oxygen, would result in an increasing surface area for microbial attachment. These findings have been reflected by the MPN counts for denitrifiers, which increased with distance from source.

In conjunction with these findings, denitrification mostly increased with distance from source, although results from the supplementary survey of the freshwater tidal reaches of the Ouse showed a decrease from the tidal limits at Naburn Weir. In order to help explain these findings, a summary of some of the main spatial trends apparent within these data for August 1996 is presented in Figure 4.16. The lowest rates of denitrification were measured at Ravenseat where, as described above, the lowest water nitrate concentrations were measured (these rarely exceeded 36 μM NO_3-N upstream of Richmond). In contrast, the highest rates of denitrification were always measured on the Wiske (concentrations as high as 2276 μM NO_3-N were recorded in January 1996). Since denitrifying bacteria require a ready supply of nitrate, as discussed previously (Section 1.21), it is probable that nitrate is a key influencing factor on denitrification in the Swale-Ouse system, furthermore the results of the statistical analyses also serve to verify this.

From the findings of the study, it would also seem apparent that sediment is potentially a key variable affecting denitrification rate, since it also varies greatly from upstream to downstream as discussed above. The rate of denitrification was found to increase as the percentages of silt and carbon increase. As previously suggested, this increase in rate could be a result of increased amounts of denitrifying bacteria due to the

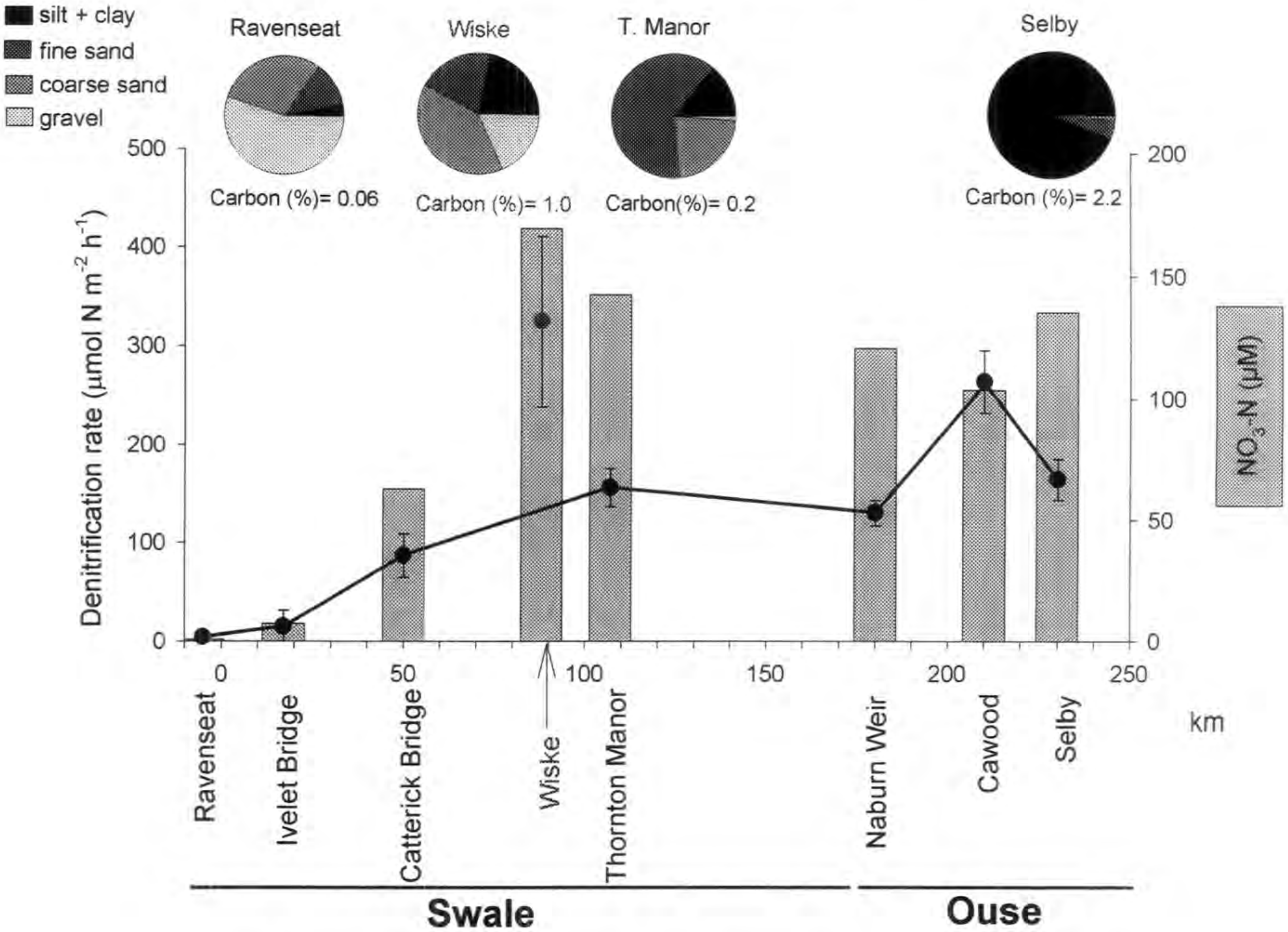


Figure 4.16 Summary of the main spatial trends along the Swale-Ouse system (August 1996) Bars indicate nitrate concentration.

increased surface area, furthermore decreased oxygen penetration would also provide more favourable conditions for denitrification (see Section 1.21). Subsequently, instability in the upper sediment due to high flow conditions could explain the decrease in denitrification in the freshwater tidal reaches downstream of Naburn Weir (Section 4.5), since this would prevent the development of microbial communities and possibly result in the greater penetration of dissolved oxygen.

The study of spatial heterogeneity also serves to reiterate the importance of sediment type on rate of denitrification, since denitrification rate showed a four-fold difference in rate between different types of sediment substrate from one site (Section 4.31). In fact, the highest rates of denitrification in this heterogeneity study were found in areas with overlying plant debris (Table 4.6) and silt. This again suggests that a higher surface area for microbial attachment, as well as potentially an increased amount of organic C content, has resulted in the higher rates of denitrification.

Denitrification also showed a seasonal trend in activity, with a peak in rate being found in the spring months for all the downstream sites. It could be hypothesised that this spring peak can be largely explained by two environmental factors, nitrate concentration in the water column and temperature. This is because the results of the best subsets regression of all water and sediment variables analysed over the long-term study period found that water nitrate and temperature explained 68 % of the total variance in the data. As discussed above, there are very marked seasonal trends evident in water column nitrate concentrations and temperature. The winter peak in nitrate could be explained by increased run off, whereas the summer low could be the result of increased macrophytic uptake and decreased run off, obviously there is a summer peak in temperature. From these observations it might appear probable that denitrification is affected by the varying importance of nitrate and temperature at different times of year. During the winter months, despite the high nitrate availability, denitrification may be expected to be limited by low temperatures, whereas during the summer months it could become limited by nitrate availability. During the spring, however, denitrification is favoured by rapidly rising temperatures whilst nitrate availability is still relatively high, thereby resulting in the observed spring peak in activity.

Similarly to denitrification, the study on N_2O production showed obvious spatial changes, increasing in production with distance from source and again exhibiting a peak on the Wiske (Section 4.4). It would also seem probable that increasing nitrate with distance from source is again a key factor influencing the tendency for this increase in N_2O production (Table 4.9 and Figure 4.13).

Nitrous oxide production showed a less obvious seasonal trend than denitrification, although there was a tendency toward higher values of N_2O production in the late spring and summer months rather than in the winter for the downstream sites, similarly to denitrification. Once again, it seems probable that higher temperatures in the summer are responsible for the generally higher rates of N_2O production then, despite the relatively low nitrate concentrations. In addition to the direct influence of higher temperature on microbial processes, a rise in temperature may enhance N_2O production by reducing oxygen concentration in the sediments, which in turn would lead to a reduction in nitrification (another source of N_2O, as well as a source of nitrate for denitrification; see Section 1.2). The decrease in N_2O production in the winter months despite the high concentrations of nitrate could be explained by low temperatures which limit denitrification. Furthermore, the instability of sediments during frequent high winter flows may also result in a decrease in denitrification and nitrification, and hence N_2O production.

The proportion of N_2O release related to the total gases released during denitrification showed great variation, again there was a seasonal trend towards higher proportions in summer months compared to winter months. This may be due to the fact that other factors, such as dissolved oxygen and organic C, content may also have important influences on N_2O production.

This discussion has provided a review of the key findings of the investigation on the Swale-Ouse system, which are further discussed in Chapter 7. However, these results do serve to support the need for further investigations into the relationship between denitrification and environmental variables.

CHAPTER 5

RIVER TWEED FIELD STUDY

5.1 Introduction

As discussed in Section 2.2, the Tweed has a rural, sparsely populated catchment with low levels of agricultural and industrial activities, resulting in low pollutant loads. For this reason, the Tweed was selected as a secondary sampling catchment to provide an 'unpolluted' contrast to the Swale-Ouse system in order to further investigate the effect of environmental variables on denitrification. Furthermore, since the Tweed was known to show consistent changes in a range of environmental variables on passing downstream (Robson *et al.*, 1996), it was hypothesised that the data collected could serve to verify the spatial and seasonal trends already observed in the Swale-Ouse system.

In order to achieve these objectives, a seasonal study from June 1996 to April 1997 was undertaken. Denitrification and environmental variable measurements at the Tweed sample sites were taken on only four occasions within the year (Section 2.22), so, there are insufficient data to allow meaningful statistical analyses. However, as these data were collected on a seasonal basis, it is still possible to utilise them to provide an inductive comparison with the main trends observed in the Swale-Ouse long-term study, even without statistical analysis.

5.2 Environment

To illustrate the environment of the Tweed during the period of the study and to help provide a better understanding of the trends evident in the denitrification data, environmental data from the LOIS core programme from August 1994 to February 1997 are presented below.

5.21 Physical variables

Temperature and flow both showed a clear seasonal bias (Fig.5.1). Flow increased with distance downstream, exhibiting a winter peak at the most upstream site at Bolside (km 73.1) and on the River Teviot at Ormiston Mill (km -9.0, 108.5). Flow on the tributary, River Teviot, was less than the main river sites, although the same seasonality was exhibited.

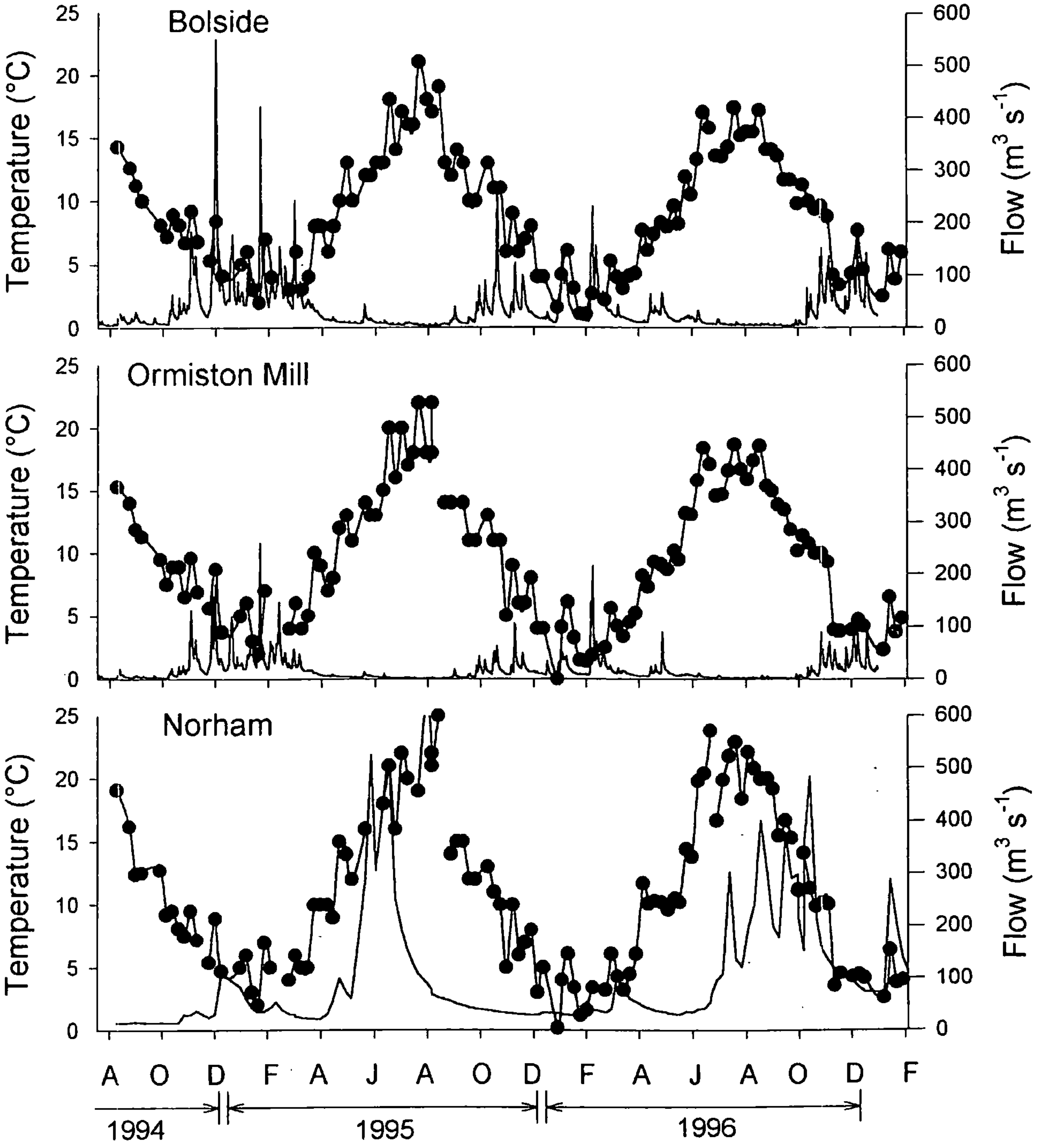


Figure 5.1 Water temperature (closed circles) and flow measurements (line) in the Tweed system from 1994 to 1997. Data from LOIS core programme.

Conductivity showed a slight increase with distance downstream (Table 5.1), although the highest values were recorded on the Teviot at Ormiston Mill, ranging from 156 to 636 μS cm^{-1}. There was no clear seasonality evident within the data.

Alkalinity reflected the conductivity, with the highest value (196.4 mg L^{-1} HCO$_3$) recorded on the Teviot at Ormiston Mill, and the lowest mean value (54.2 mg L^{-1} HCO$_3$) being calculated for the most upstream site at Bolside (Table 5.1).

The average pH values between sites on the Tweed were not greatly different (e.g. 8.2 at Ormiston Mill, Table 5.1). However, the data at Norham showed much greater variation than the other sites, often becoming highly alkaline in the summer months and more acid in the winter months (Fig. 5.2).

Table 5.1 Means and ranges of water variables in the Tweed river system (measured on day of sediment sampling) from August 1995 to August 1996. Cond. = Conductivity; Alk. = Total alkalinity.

	Bolside			**Ormiston Mill**			**Norham**		
km distance	73.1			-9.0, 108.5			138.3		
	mean	min.	max.	**mean**	min.	max.	**mean**	min.	max.
Cond. (μS cm^{-1})	**213**	117	407	**339**	156	636	**265**	155	539
pH	**8.6**	6.7	10.0	**8.2**	7.0	9.4	**8.4**	6.9	10.5
Alk. (mg L^{-1} HCO$_3$)	**54.2**	15.7	192.8	**137.6**	10.7	196.4	**85.0**	4.9	190.7

5.22 Water chemistry variables

Nitrogen fractions

Nitrate concentration in water showed a slight increase with distance downstream (Figure 5.3). Ormiston Mill on the Teviot generally had maximum concentrations. A very clear seasonal trend was evident in the nitrate data for all sites, with the highest values being measured in the winter and early spring.

Nitrite and ammonium showed no clear seasonal trends (Figure 5.4). Nitrite rarely exceeded 1.5 μM in the Tweed system, although the highest values were usually measured at Ormiston Mill, where a maximum of 11 μM was recorded in September 1996. Ammonium concentrations on the Tweed were usually below 10 μM, often with higher measurements on the upstream site at Bolside, possibly due to input from a sewage treatment works just upstream.

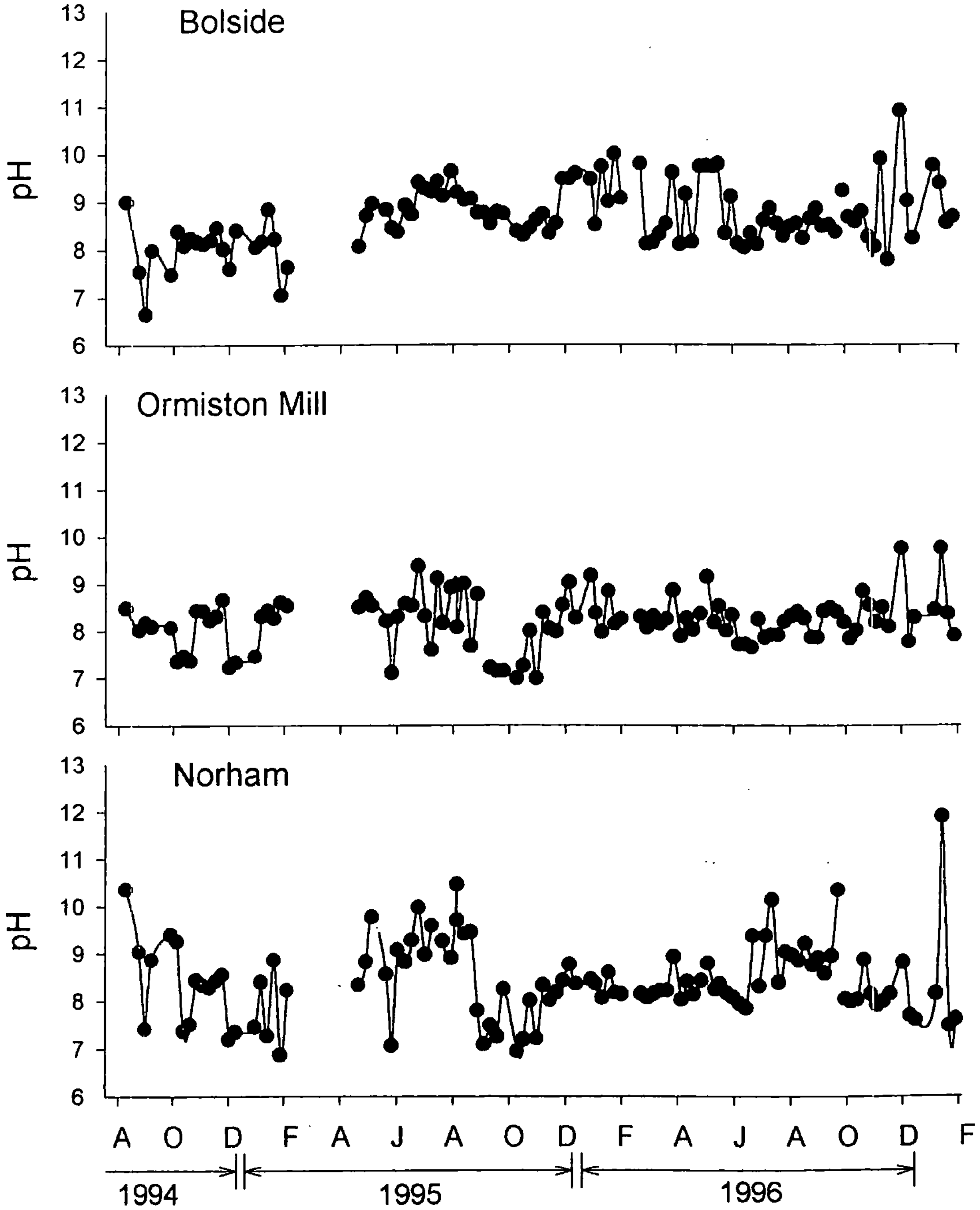


Figure 5.2 pH measurements in water measured in the Tweed system from 1994 to 1997. Data from LOIS core programme.

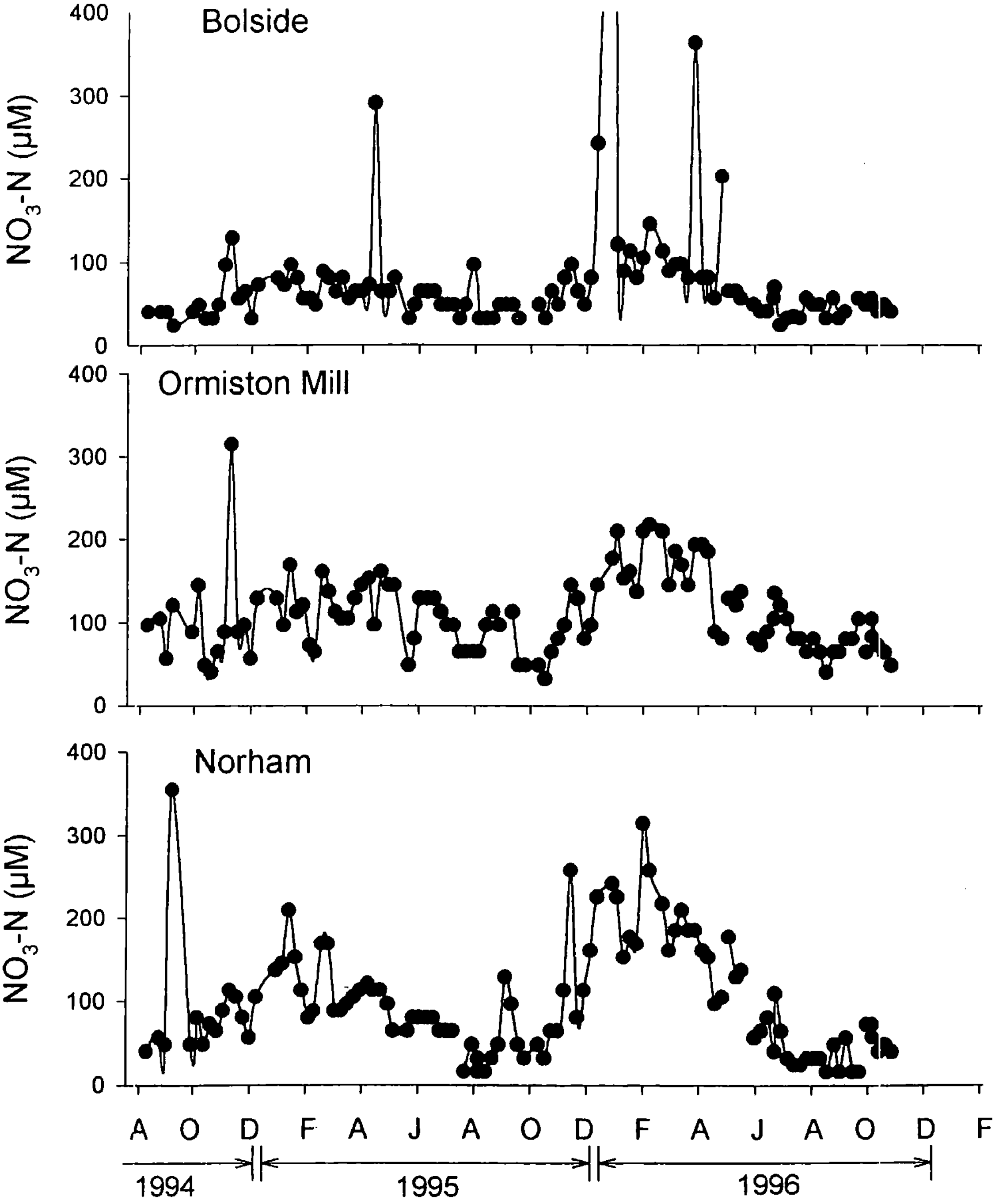


Figure 5.3 NO_3-N concentrations in water measured in the Tweed system between 1994 and 1996. Data from LOIS core programme.

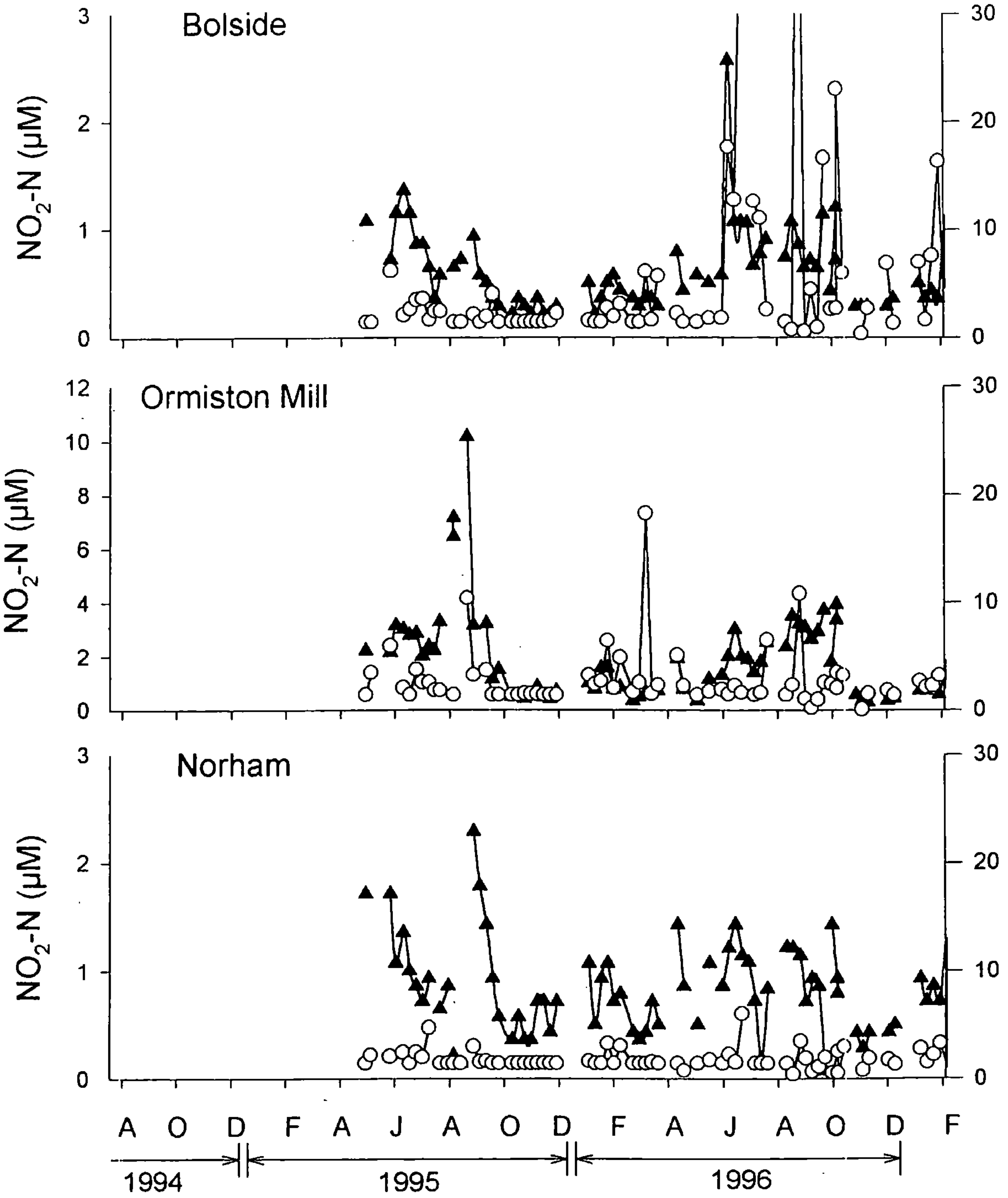


Figure 5.4 NO$_2$-N (closed triangle) and NH$_4$-N (open circle)concentrations in water measured in the Tweed system between 1995 and 1997. Data from LOIS core progra,mme.

Phosphorus fractions

Average aqueous phosphorus concentrations were low (Table 5.2), both organic and inorganic forms were close to detection limits at certain times of year. The highest mean concentrations for all fractions were measured at Ormiston Mill.

Table 5.2 Means and ranges of phosphorus fractions in the Tweed river system (measured on day of sediment sampling) from August 1995 to August 1996. FRP = Filtrable reactive phosphorus; FTP = Filtrable total phosphate; TP = Total phosphate.

	Bolside			**Ormiston Mill**			**Norham**		
km Distance	73.1			-9.0, 108.5			138.3		
	mean	min.	max.	**mean**	min.	max.	**mean**	min.	max.
FRP ($\mu g\ L^{-1}$ P)	**38**	4	272	**35**	4	128	**30**	5	77
FTP ($\mu g\ L^{-1}$ P)	**62**	9	330	**91**	3	1188	**81**	10	966
TP ($\mu g\ L^{-1}$ P)	**110**	22	1937	**123**	15	1224	**118**	22	989

5.3 Sediment denitrification

Denitrification rates measured using intact sediment cores in the lower Tweed are shown in Figure 5.5 to allow comparison with the background environmental data.

Spatial trend

The levels of denitrification activity for each site mirrored the levels of nitrate measured in the overlying water (Fig. 5.6), phosphorus and conductivity. The lowest rates (below 20 μmol N m^{-2} h^{-1}) were recorded at Bolside, the most upstream site, which always had the lowest nitrate measurements on day of sampling (Fig. 5.6). The highest rates (242 μmol N m^{-2} h^{-1}) were consistently found on the more polluted tributary at Ormiston Mill, which, in contrast to Bolside, always had the highest nitrate measurements on the day of sediment sampling (Fig. 5.6). Denitrification rates measured at the most downstream site (East Ord, km 150.5), in the freshwater intertidal zone of the Tweed, were always slightly lower than Norham (just upstream of the tidal limit), despite the fact levels of nitrate were very similar.

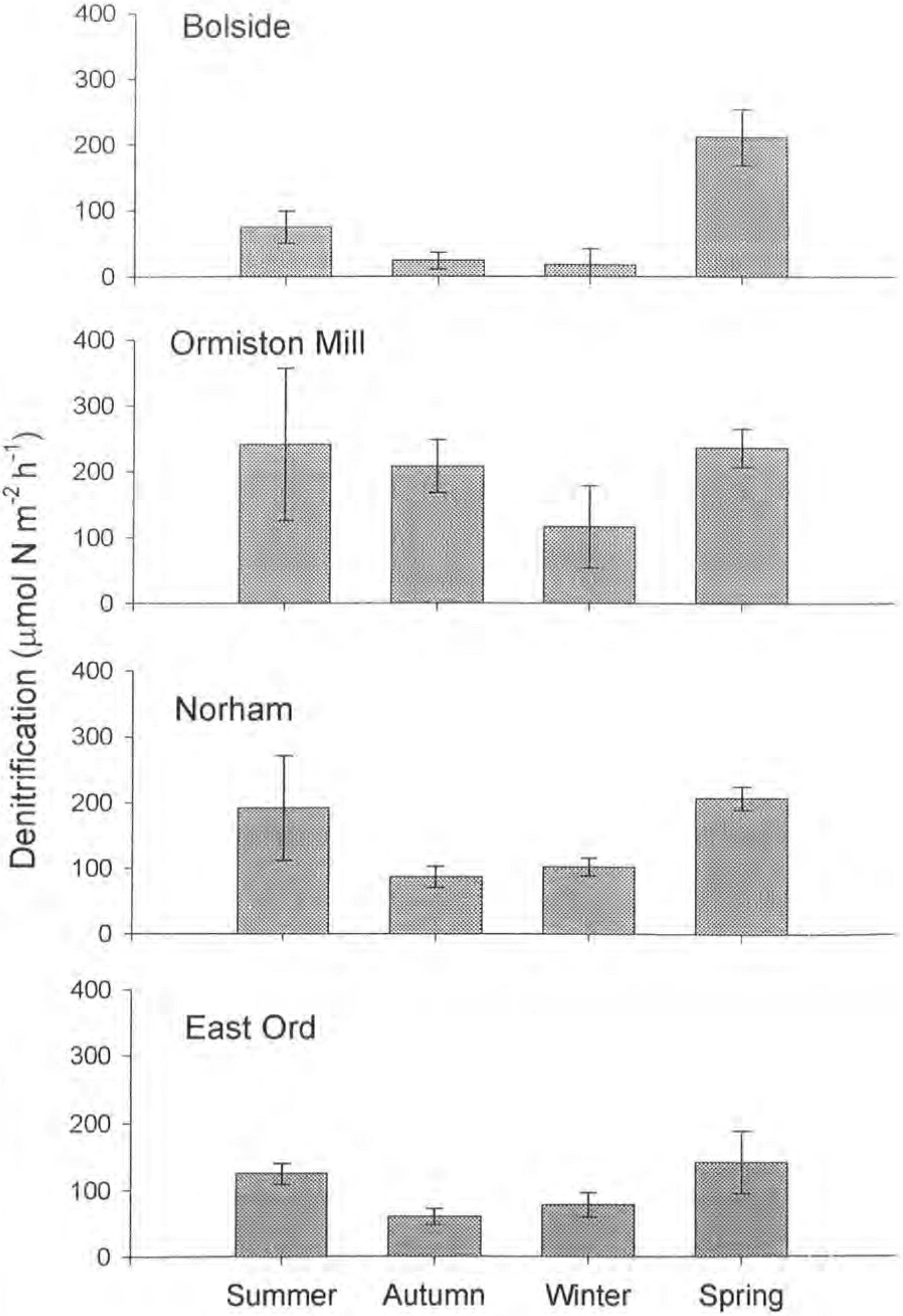


Figure 5.5 Denitrification rates in intact sediment cores obtained seasonally in the Tweed system from 1996 to 1997. Bars denote the standard error of three replicates.

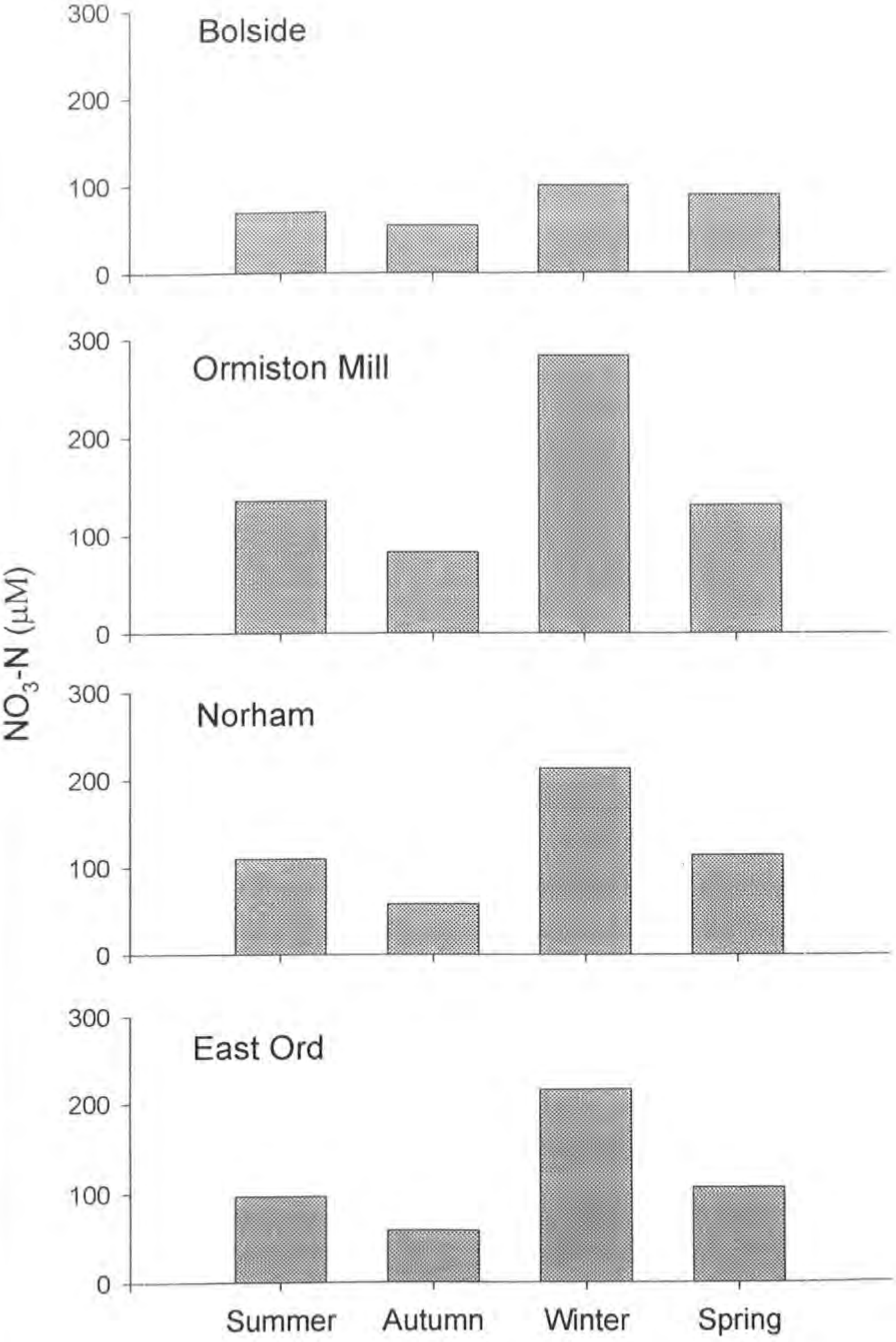


Figure 5.6 NO$_3$-N concentrations in water obtained seasonally (on each sampling occasion) in the Tweed system from 1996 to 1997.

Seasonal trend

A spring peak in denitrification activity was found on the Tweed (Fig. 5.5), which suggests a seasonal trend. For example, at Bolside a rate of 213 μmol N m^{-2} h^{-1} was recorded in April 1997 compared to 17 μmol N m^{-2} h^{-1} the previous January. Denitrification in the summer months at all sites was generally a little lower than in spring, the level of activity then troughed during the autumn and winter months.

5.4 Nitrous oxide (N_2O) production

Spatial trend

Nitrous oxide production during the assays (intact sediment cores without acetylene) ranged from a slight net uptake to a notable release (Table 5.3). Low or slightly negative values were recorded at all sites, although there was a tendency for maximum values to increase on moving downstream, with the highest values recorded on the Teviot, the lowland tributary. For example at Bolside, the values ranged from -45.0 μmol N_2O-N m^{-2} h^{-1} (i.e. net uptake) to 33.7 μmol N_2O-N m^{-2} h^{-1} (i.e. net release). Downstream at East Ord, values ranged from -0.2 to 60.8 μmol N_2O-N m^{-2} h^{-1} and for the Teviot, values ranged from -9.6 to 185.6 μmol N_2O-N m^{-2} h^{-1}.

Table 5.3 N_2O production in the sediments in the Tweed system from summer 1996 to spring 1997. Samples were collected seasonally.

River	Site	Distance (km)	N_2O production (μmol N_2O-N m^{-2} h^{-1}) min.	mean	max.
Tweed					
	Bolside	73.1	-45.0	-5.8	33.7
	Norham	138.3	4.2	26.2	56.3
	East Ord	150.5	-0.2	29.2	60.8
Teviot					
	Ormiston Mill	-9.0, 108.5	-9.6	51.4	185.6

Seasonal trend

All sites showed a tendency toward seasonal changes in nitrous oxide production (Figs. 5.7 & 5.8). An autumn/winter low was generally evident at all sites leading to a spring/summer peak, this is particularly evident at Bolside and Ormiston Mill (Fig. 5.7). The highest nitrous oxide production rate of 186 μmol N_2O-N m^{-2} h^{-1} was measured on the Teviot at Ormiston Mill on 25th June 1996.

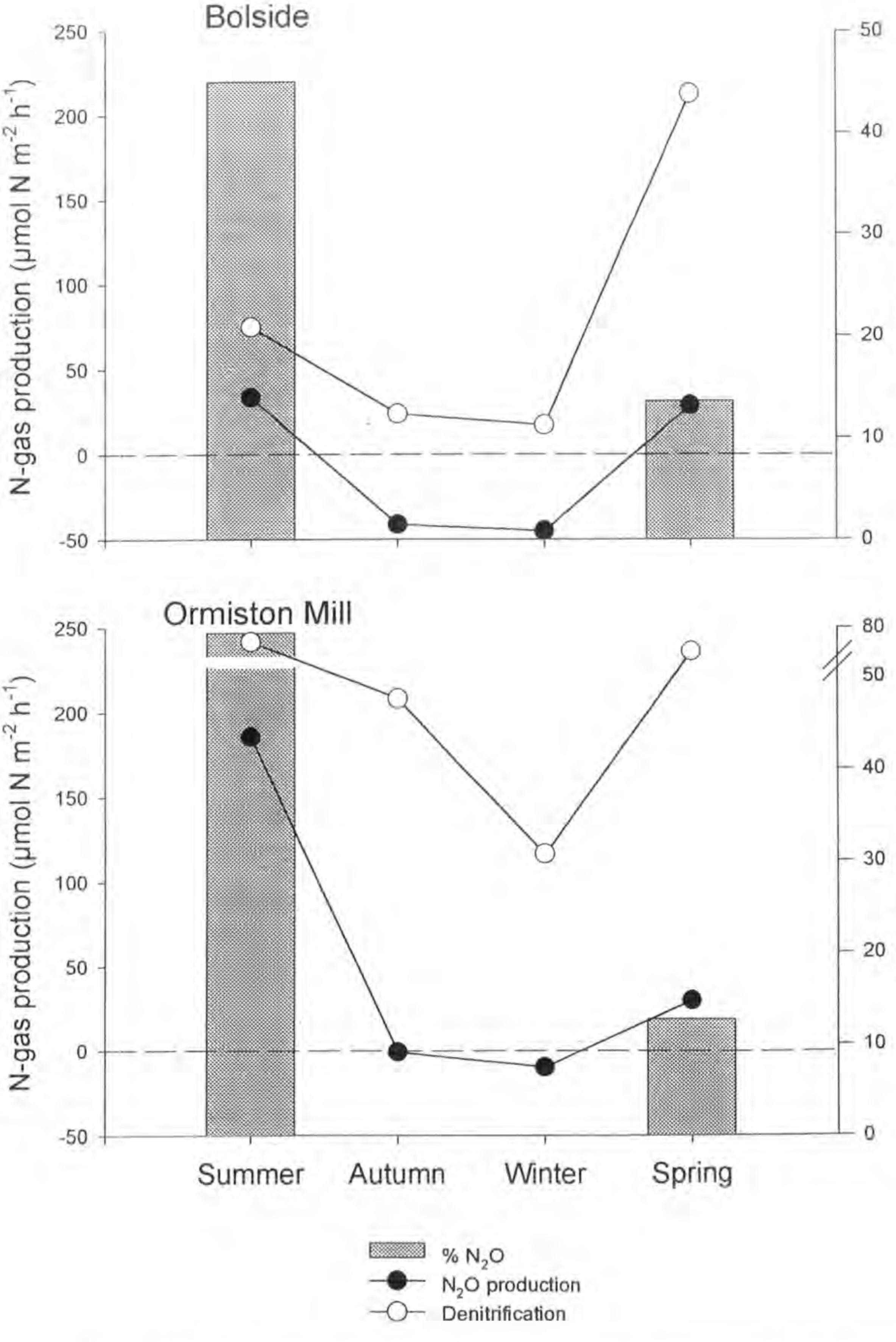


Figure 5.7 Nitrous oxide production from sediments at Bolside and Ormiston Mill in the Tweed system from 1996 to 1997.

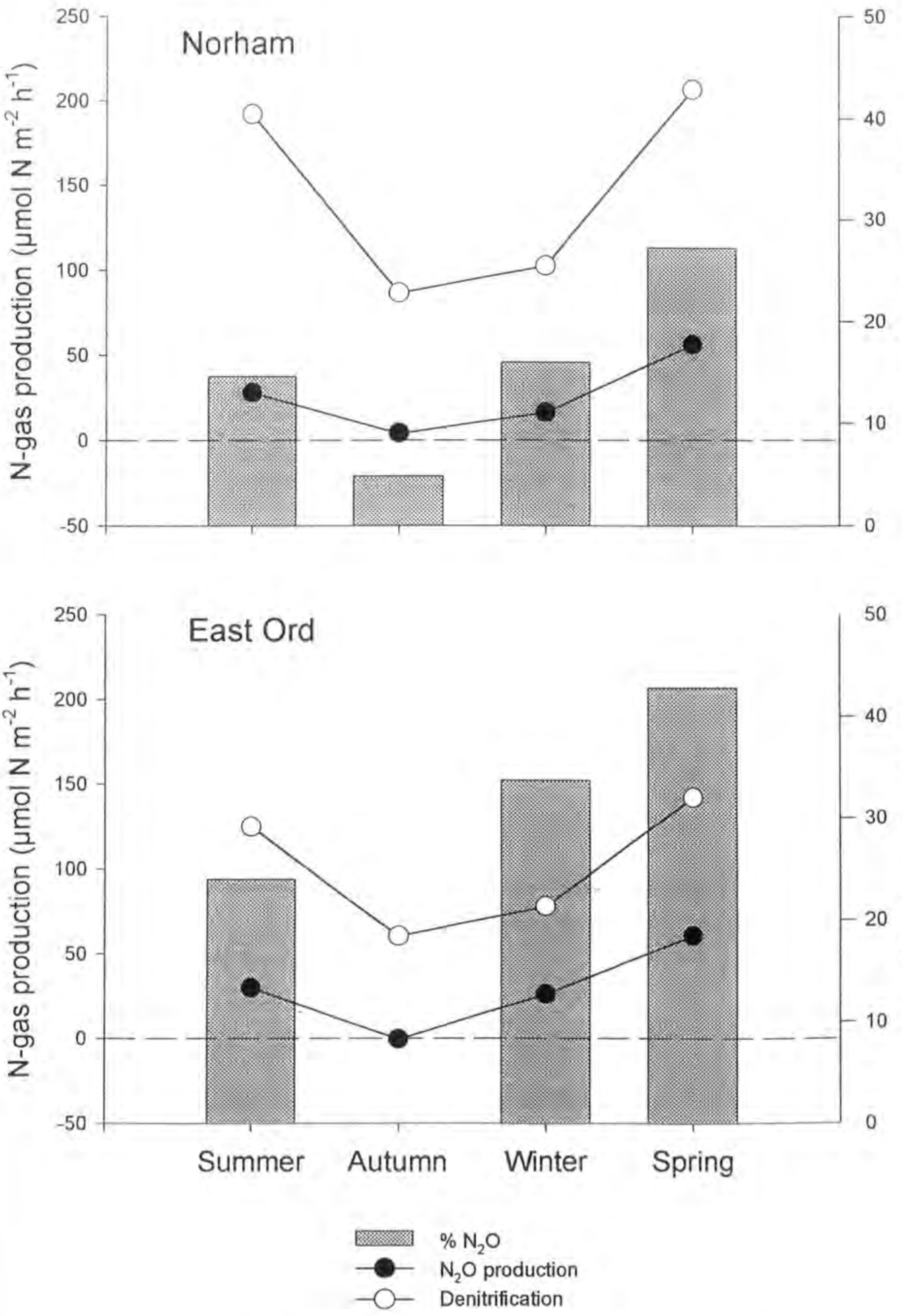


Figure 5.8 Nitrous oxide production from sediments at Norham and East Ord in the Tweed system from 1996 to 1997.

The proportion of nitrous oxide release related to the total gases released during denitrification (N_2O + N_2 from intact sediment cores with acetylene) showed great variation (minimum 0, maximum 77 %) as indicated by the bars on Figures 5.7 and 5.8. However, there was again a general trend towards higher proportions of nitrous oxide release in the spring/summer months compared to the autumn/winter months. The greatest proportion of nitrous oxide released was from sediments at Ormiston Mill on the Teviot; a maximum of 77 % was measured on 25th June 1996.

5.5 Discussion

The environmental measurements taken on the Tweed were of a similar order of magnitude to those on the Swale at Catterick Bridge (see Tables 4.1 & 4.2) in the upper reaches of the Swale-Ouse system, upstream of any significant organic inputs. This serves to confirm the low pollutant loading of the Tweed.

As previously observed in the Swale-Ouse system, seasonal and spatial trends in various environmental factors were also evident on the Tweed. Conductivity, alkalinity, pH and nutrient fractions generally increased on moving downstream, with a peak being found on the tributary, the River Teviot, probably as a result of a decreased dilution effect and inputs from its relatively lowland catchment. Flow, temperature, pH and nitrate concentration all showed seasonal fluctuations. Flow and nitrate concentration generally exhibited a winter peak, whereas temperature and pH peaked in the summer months. The summer high alkalinities, in the mid to lower reaches in particular, may be explained by biochemical processes resulting from the presence of mass algal growths (Tweed River Purification Board, 1957).

Although only four seasonal measurements of denitrification were performed at each site, they still serve to verify the spring peak in denitrification that was observed in the Swale-Ouse system (see Section 4.3). This would again potentially relate to increasing temperature and availability of nitrate. This is backed up by the fact that the rates of denitrification measured in the Tweed were of a similar order of magnitude to those measured in the middle reaches of the Swale at Catterick Bridge, which, as previously observed, also had similar levels of nitrate (see Sections 4.22 & 4.3). In addition, once again the levels of denitrification activity mirrored the levels of nitrate on moving downstream, except for the freshwater tidal site at East Ord where there was a decrease in rate, similarly to Selby on the Ouse (see Section 4.5). Furthermore, the maximum rates of denitrification were consistently recorded at Ormiston Mill on the

Teviot, the tributary on which the highest concentrations of nitrate were measured. This would again suggest the strong relationship between denitrification and environmental nitrate availability, except where other environmental factors, such as sediment stability, exerted an influence.

Nitrous oxide production generally mirrored denitrification, showing a tendency toward an increase in nitrous oxide production on moving downstream, with the maximum values being recorded on the Teviot, the tributary. It seems probable that increasing nitrate concentration in the water is an important factor influencing this trend. The minimum in nitrous oxide production during the autumn/winter months for all sites and a peak in production during the spring/summer months, is probably a result of increased ambient temperatures. This trend was also observed in the Swale-Ouse study (see Section 4.4). Furthermore, the proportion of nitrous oxide production related to the total gases released during denitrification also showed a trend towards higher proportions in the summer months compared to winter months.

It would appear that the findings from this study on the Tweed can confirm the hypothesis that a gradient of physical and environmental variables along a river continuum can elicit a graded response from the denitrifying bacterial populations (see Section 1.4). It has also been suggested by this study that as these environmental factors vary seasonally, so does the rate of denitrification. The relative influence of these regulating environmental factors is further explored in the next chapter.

CHAPTER 6

FACTORS AFFECTING DENITRIFICATION

6.1 Introduction

The previous chapters (Chapters 4 & 5) have demonstrated that the physical and chemical variables of water and sediment present a gradient from the headwaters to the estuary, which has, in turn, elicited a response from the denitrifying bacterial population. These observations serve to verify the river continuum concept of Vannote *et al.* (1980); for example, in the Swale-Ouse a consistent increase (up to 30 times) in denitrification rate of sediment cores has been recorded on passing downstream. In addition to this, the seasonal variation in the influencing environmental factors has also elicited a response in denitrifying bacteria, therefore resulting in the seasonal trends observed in denitrification rate in both the Swale-Ouse and Tweed river systems.

Thus, through these long-term studies presented in the previous chapters, it has been established that denitrification is affected, both seasonally and spatially, by a variety of environmental parameters, thereby confirming the first two original hypotheses (Section 1.4). Therefore, it can be hypothesised that some environmental parameters may exert a greater influence on denitrification than others, thereby resulting in the observed seasonal and spatial trends (Section 1.4). In order to achieve this, it was decided to further investigate the relationship between denitrification rate in sediments and the chemical and physical characteristics of water and sediments through intensive field and laboratory short-term investigations.

6.2 Field investigation

6.21 Introduction

The aim of this study was to examine the relationship between potential denitrification rate and N_2O production in sediments and the chemical and physical characteristics of water and sediments for a large number of rivers during one season and, as far as possible, one set of climatic conditions. This would then help to establish which environmental parameters affected denitrification to the greatest extent throughout a series of river systems rather than just one, as in the previous chapters. Therefore, this may help to confirm the findings of the long-term studies.

To achieve this, 50 sites were selected from 30 rivers in N-E. England

(Table 6.1); all sites on any one river were separated by at least a 5 km river distance. The rivers ranged from small second-order tributaries to moderately large sixth-order rivers, and the width from about 1 to 30 m. The study was carried out after several months of relatively low river flows and the water depth was usually < 1 m at the time of sampling. Many of the sites had been the subject of previous studies and were selected to show a wide range of physical and chemical properties (Table 6.2). Sediment samples (0-5 cm depth) were collected over a 3-week period in September 1996.

Table 6.1 Location of the sampling sites for the 50 river sites survey (September 1996), including the grid reference.

River	grid ref.	River	grid ref.	River	grid ref.
Swale	NY863032	Derwent	SE706512	Seven	SE745854
	SD934977	Foss	SE622592	East Allen	NY846558
	SE226994	Nidd	SE483562	South Tyne	NZ844644
	SE434715	Cod Beck	SE411761	North Tyne	NZ919705
Ouse	SE558551	Gaunless	NZ184266	Browney	NZ221455
	SE598456	Skerne	NZ287125		NZ225453
	SE575370	Wharfe	NZ455094	Wiske	SE376845
	SE626329	North Burn	NZ453269	Deerness	NZ226422
Tees	NY853312	Pont	NZ168743	Deerness	NZ226422
	NY996233	Blyth	NZ190777		
	NZ168160	Coquet	NU185004		
	NZ331102	Font	NZ172860		
	NZ449162	Wansbeck	NZ195864		
Tyne	NZ989643	Derwent	NZ077502		
	NZ095622		NZ198624		
Wear	NY888382	Ure	SE333703		
	NZ074368	Rye	SE615836		
	NZ288411		SE796753		
	NZ285523	Hodge Beck	SE680850		
	SE524401	Dove	SE790863		

Table 6.2 Pertinent sample statistics for the various water and sediment variables from the 50 river sites survey (September 1996). From 0 to 5 cm depth

Variables	Units	Max.	Min.	Mean
Water				
NO_3-N	$\mu g\ L^{-1}$	8174	3.4	1935
NO_2-N	$\mu g\ L^{-1}$	344	0	64.6
NH_4-N	$\mu g\ L^{-1}$	4277	14.3	243
PO_4-P	$\mu g\ L^{-1}$	4658	21.6	870
Conductivity	$\mu S\ cm^{-1}$	1557	116	531
Sediment				
C (<100 µm)	%	7.3	<0.01	1.11
N (<100 µm)	%	0.51	<0.001	0.06
C/N ratio		48.4	11.2	18.6
Water content	%	86.6	16	38.4
Particle sizes				
<100 µm	%	95.1	0.10	26.8
100 -150 µm	%	20.8	0.25	6.68
150- 250 µm	%	50.3	0.30	20
250- 600 µm	%	73.1	0.05	26.1
>600 µm	%	93.9	0.03	19.5

6.22 Results

Environmental features of the water and sediments are shown in Fig. 6.1. Nitrate concentration ranged from 0.24 to 584 µM with 36 % of samples less than 50 µM (Fig. 6.2). Water content of the sediments was distributed normally and ranged from 16 to 86 %. The percentage of was low in upland sites and usually increased on passing downstream, the highest values (up to 95 %) occurring at a tidal (freshwater) site on the Ouse at Selby. 52 % of samples showed values under 20% particles < 100 µm.

The initial N_2O concentration in sediment ranged from 0.005 to 19.7 nmol N_2O-N g^{-1} d. wt (R. Gaunless), although 90 % of river sediments were below 2 nmol N_2O-N g^{-1} d. wt (Fig. 6.4a). N_2O concentration showed a highly skewed distribution, with many low values and a few high values. The accumulation of N_2O in the samples with no added acetylene ranged from negative values (indicating net

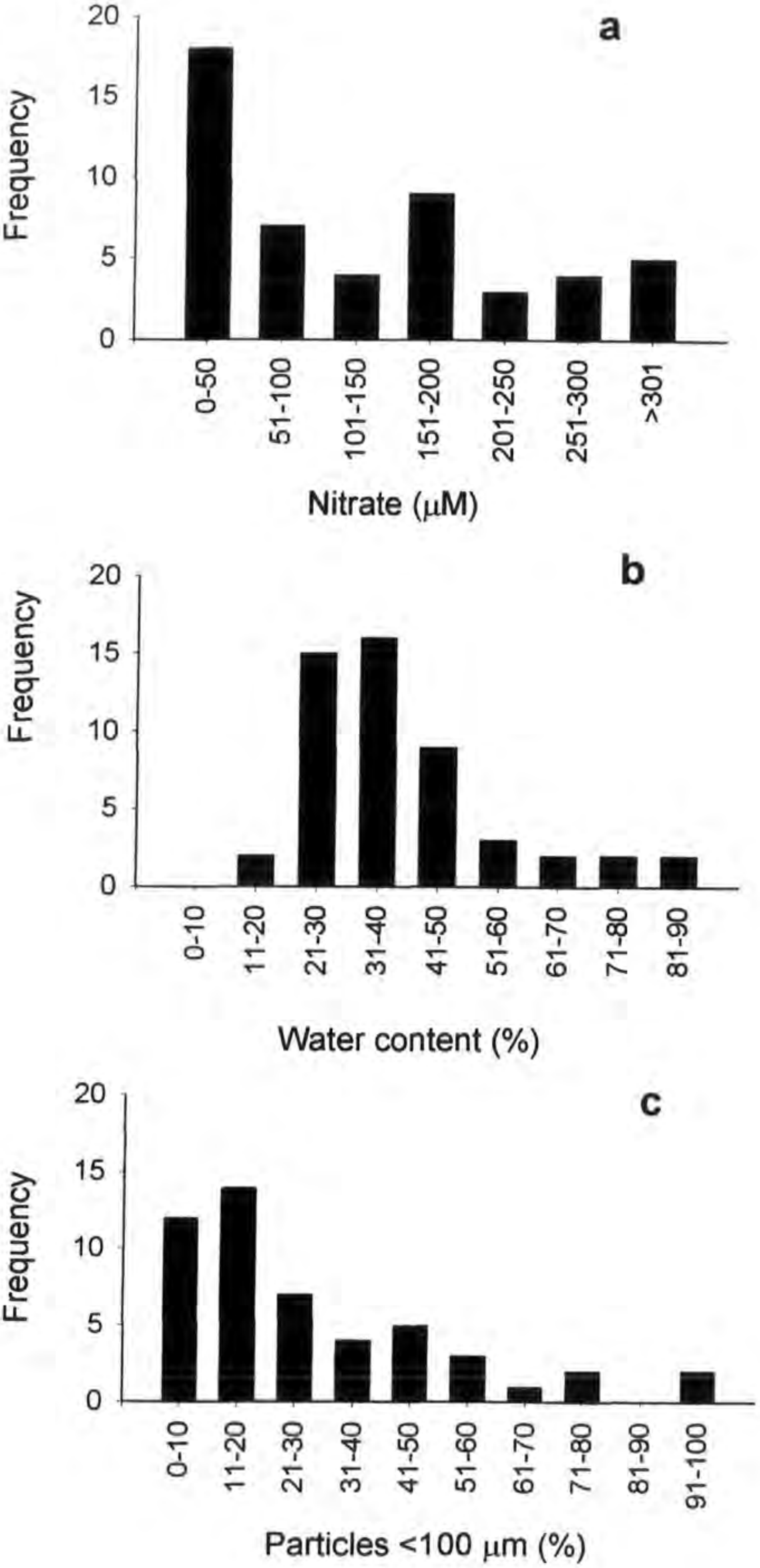


Figure 6.1 **Frequency distribution of river nitrate (a), water content in the sediment (b) and percentage of particles < 100 μm (c) in the 50 river sites survey (September 1996).**

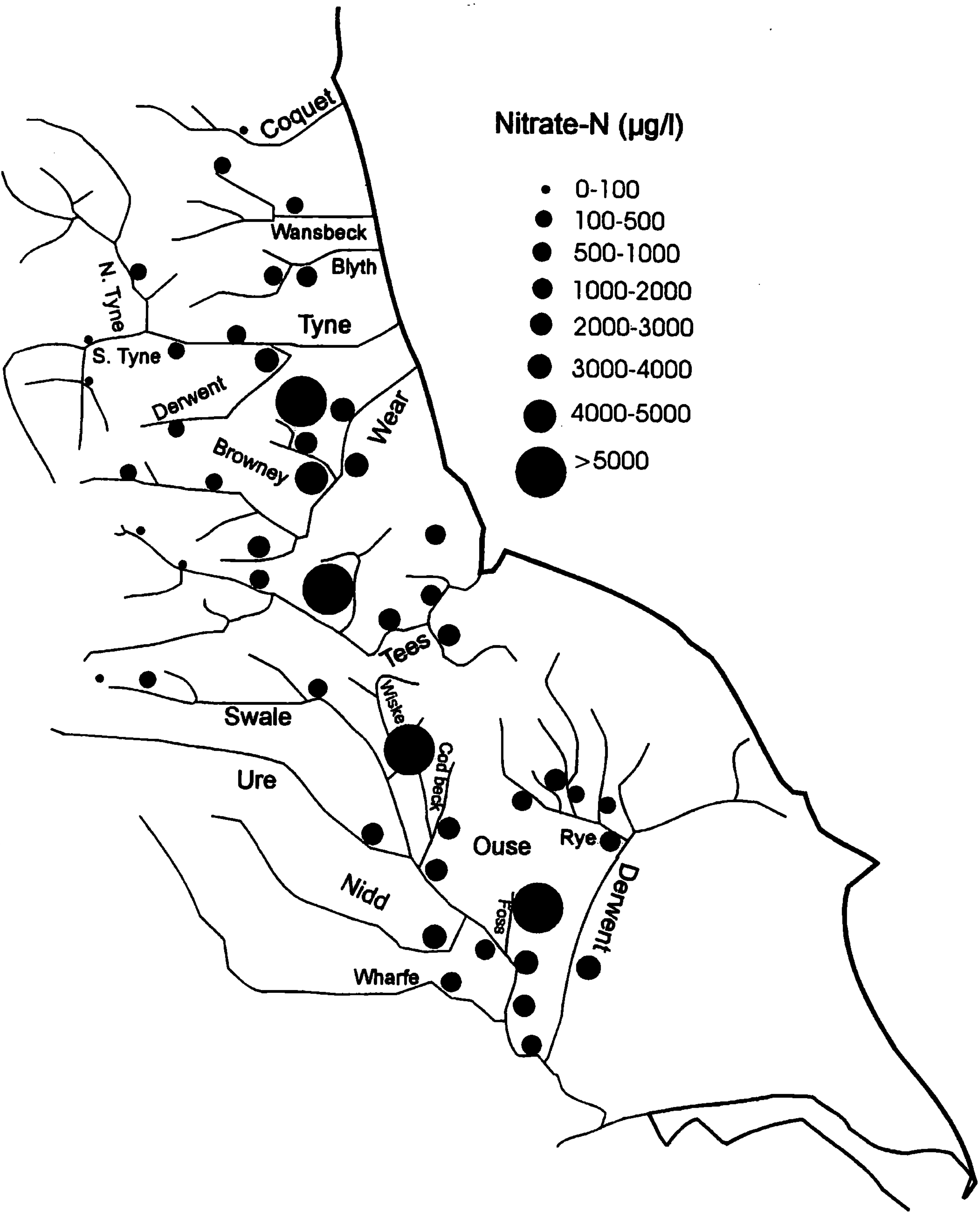


Figure 6.2 Nitrate concentration in water of the investigated rivers for the 50 river sites survey (September 1996).

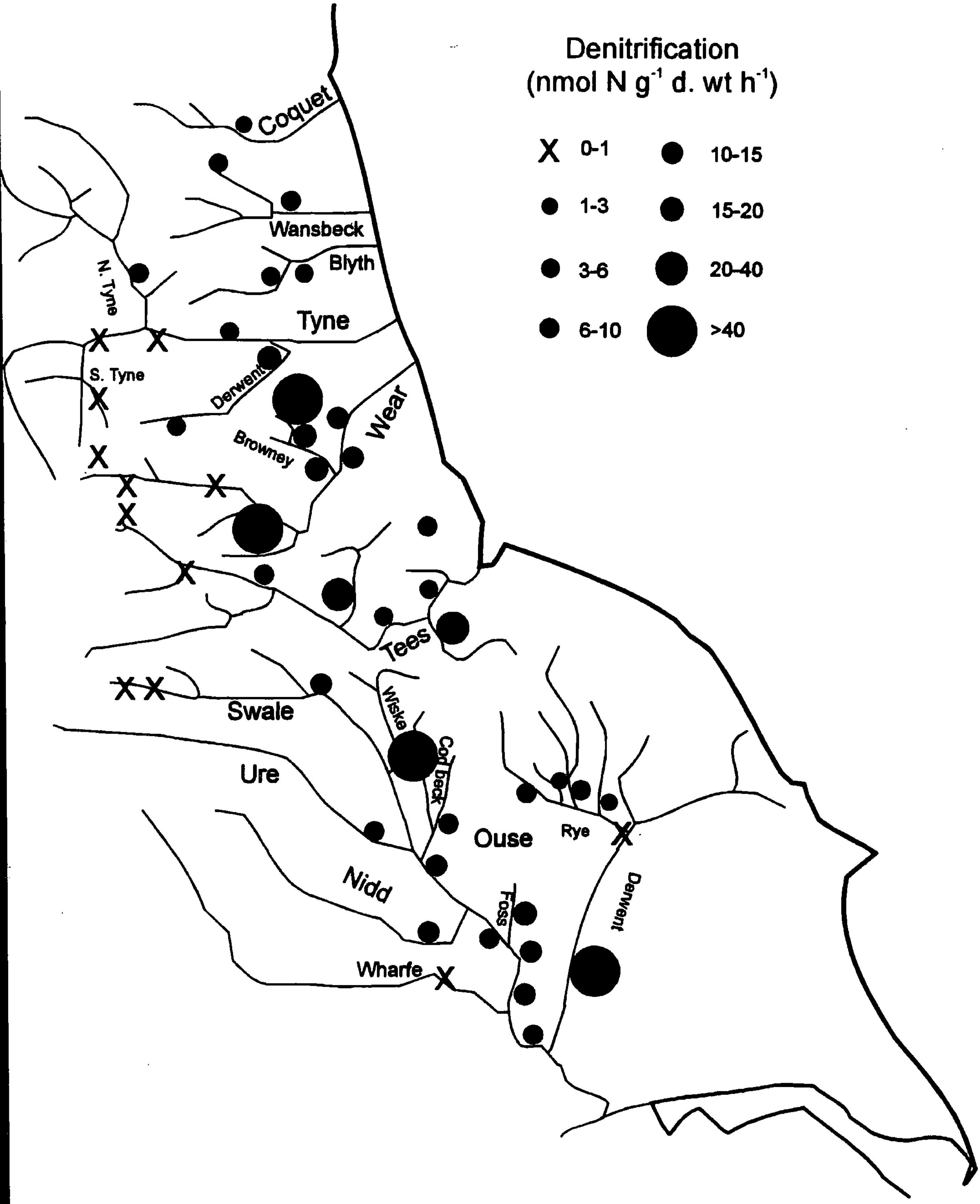


Figure 6.3 Potential denitrification measured in sediment from the investigated rivers of the 50 river sites survey (September 1996).

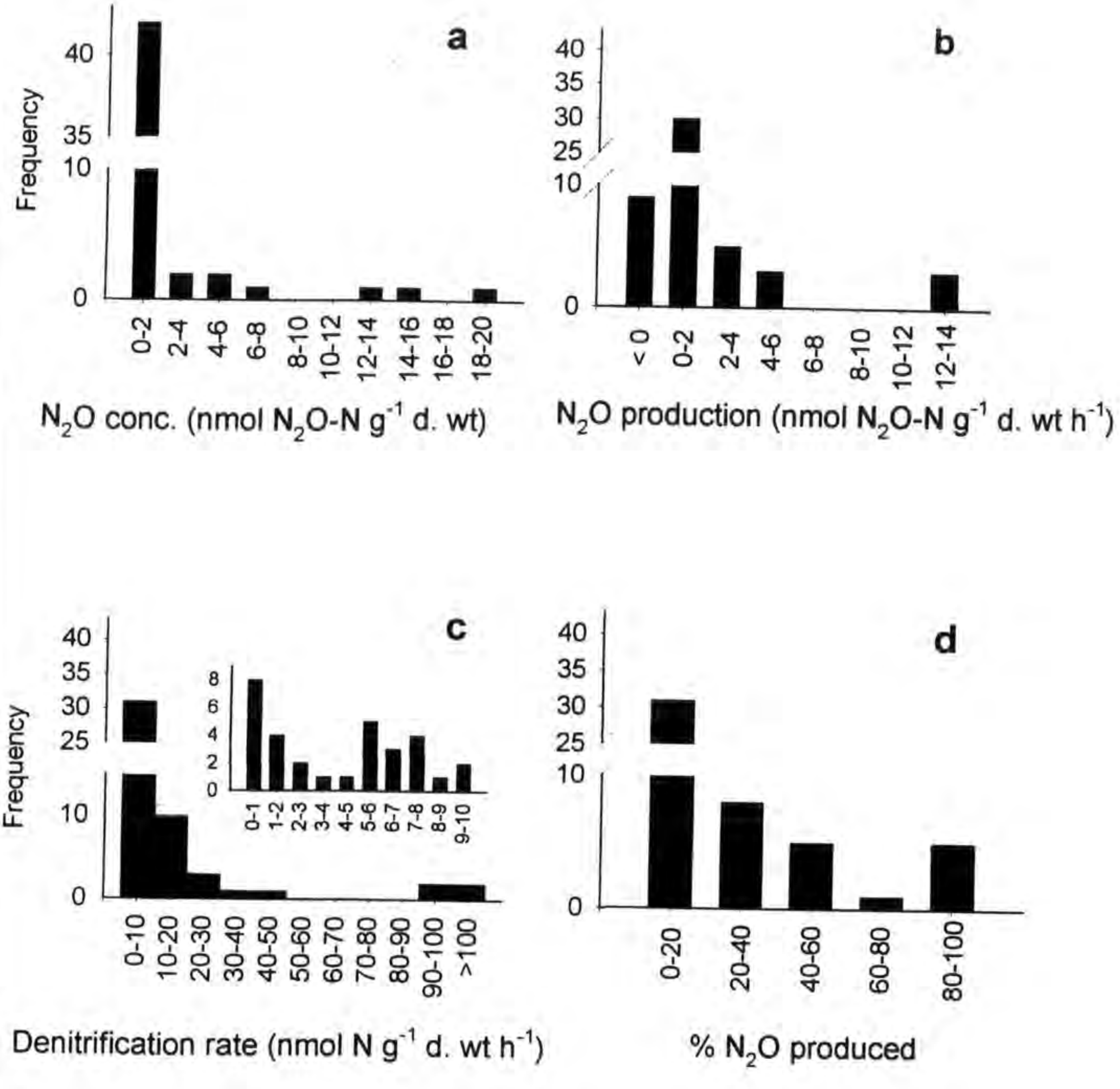


Figure 6.4 Frequency distributions of nitrous oxide concentration in the sediment (a), nitrous oxide production (b), potential denitrification rate (c) and percentage of nitrous oxide produced (d) in the 50 river sites survey (September 1996).

consumption of N_2O during the assay) to 13.1 nmol N_2O-N g^{-1} d. wt h^{-1} (Fig. 6.4b). 90 % of samples showed rates less than 4 nmol N_2O-N g^{-1} d. wt h^{-1}.

The denitrification rate ($N_2 + N_2O$ production) in the various sediments is shown in Figures 6.3 and 6.4c. The rate ranged from below the detection limit at Ravenseat (tributary of R. Swale) to 260 ± 11 nmol N g^{-1} d. wt h^{-1} in R. Deerness near Durham. 90 % of the sediments analysed had a rate lower than 40 nmol N g^{-1} d. wt h^{-1}. Only sediment from the Deerness, Gaunless, Kexby and Wiske showed rates above this value.

Denitrification rates on passing down a particular river showed increasing values (Fig. 6.3). For instance, the rates increased from below detection limit to 12.5 ± 1.4 (R. Swale), 0.5 ± 0.04 to 5.6 ± 0.8 (R. Tees) and 1.5 ± 0.15 to 14.7 ± 1.7 nmol N g^{-1} d. wt h^{-1} (R. Wear).

The proportion of N_2O related to total gases ($N_2O + N_2$) ranged from 0.1 to 115 %, and 38 % of these samples produced over 20 % N_2O to total gases, Fig. 6.4d.

Correlation analysis

In order to help deduce the relative influence of each environmental variable on potential denitrification rate and nitrous oxide production, a correlation analysis was performed on the data.

The log transformed concentration of N_2O in the sediments was significantly and positively correlated ($p<0.001$) with the water content of the sediment and percentage values for total C and N in the sediment. No significant negative correlation was calculated (Table 6.3). The production of N_2O in those samples without acetylene was positively correlated, although weakly, with conductivity, nitrate and phosphate in the river water (Table 6.4). Denitrification rate showed a strong positive correlation with water content of the sediments and percentage values for sediment C and N and percentages of particles smaller than 100 µm (Table 6.3). When correlated with water variables, denitrification rate also showed strong positive correlation with conductivity, alkalinity, nitrate and phosphate (Table 6.4). Denitrification rate did not show a significant negative correlation with any variable.

Many of the sediment variables analysed showed strong interrelationships. Thus, water content was strongly positively correlated with C and N content and more weakly with the proportion of particles smaller than 100 µm (Table 6.3). In the case of the water variables, nitrate was strongly positively correlated with conductivity and phosphate and weakly with alkalinity and nitrite (Table 6.4)

Table 6.3 Pearson correlation coefficients for N_2O concentration in the sediment (N_2O init.), N_2O production (N_2O prod.), potential denitrification (DR) and determinant sediment variables for the 50 river sites survey (September 1996). Only values over 0.44 ($p<0.001$) are shown. All variables were log transformed except for water content and C:N ratio. N_2O production was log (x+1) transformed. P1, P2, P3, P4 and P5 correspond to the percentage of particles smaller than 100 μm, between 100-150, 150-250, 250-600 and > 600 μm respectively.

Variable	**% H_2O**	**% C**	**% N**	**C:N**	**P1**	**P2**	**P3**	**P4**	**P5**
% H_2O									
% C	0.69								
% N	0.79	0.96							
C:N	-	-	-						
P1 (%)	0.44	0.89	0.78	-					
P2 (%)	-	-	-	-	-				
P3 (%)	-	-	-	-	-	0.83			
P4 (%)	-	-	-	-0.47	-	-	-		
P5 (%)	-	-	-	-	-0.54	-	-	0.55	
N_2O init.	0.64	0.47	0.49	-	-	-	-	-	-
N_2O prod.	-	-	-	-	-	-	-	-	-
DR	0.49	0.50	0.51	-	0.44	-	-	-	-

Table 6.4 Pearson correlation coefficients for N_2O concentration in the sediment (N_2O init.), N_2O production (N_2O prod.) and potential denitrification (DR) and determinant water variables for the 50 river sites survey (September 1996). Only values over 0.44 ($p<0.001$) are shown. All variables were log transformed except for nitrous oxide production (log (x+1) was applied).

Variables	NO_3-N	NO_2-N	NH_4-N	PO_4-P	Cond.	Alk.
NO_3-N						
NO_2-N	0.57					
NH_4-N	-	0.46				
PO_4-P	0.72	0.62	-			
Conductivity	0.66	0.61	-	0.74		
Alkalinity	0.50	0.51	-	0.50	0.89	
N_2O init.	-	-	-	-	-	-
N_2O prod.	-	-	-	-	-	-
DR	0.73	0.46	-	0.58	0.64	0.56

Multiple regression analysis

A multiple regression analysis was performed in order to establish which water and sediment variables exerted the greatest combined influence on potential denitrification rate and nitrous oxide production.

In order to obtain normality for the variables, values for all except water content and the ratio for sediment C:N were transformed. Transformation involved log (x) application, except for N_2O production, which showed several negative values, thus a log (x+1) transformation was applied. When all the variables analysed were computed, the multiple regression analysis explained 77 % of all the variation in the denitrification data ($P < 0.001$), Table 6.5.

Table 6.5 Multiple regression model of potential denitrification rate (log transformed) versus sediment and water variables in 50 river sites survey (September 1996) analysis.

Variables	Significance	$R^{2\#}$
Water column nitrate (μM)	$P<0.001$	0.53
Sediment water content (H_2O %)	$P<0.001$	0.64
All variables	$P<0.001$	0.77

#Cumulative proportion of variation in denitrification rate that can be accounted for by each variable and all preceding variables

However, some of these variables may be unrelated or only weakly related to denitrification rate or contain information already provided by other predictors. A model with many predictors can also be cumbersome to use and difficult to interpret and reduce the number of degrees of freedom, leading to a deterioration in the degree of precision. In order to reduce the number of variables, a stepwise statistical procedure was used. It was calculated that with two variables, nitrate in the water and water content of sediments, 64 % of all variation in the values for denitrification rate could be explained ($p<0.001$) (Table 6.5). The relationship between nitrate in the water column and water content of sediments is described in Equation 1.

$$\mathrm{Log}\ (N_2+N_2O)\text{-}N\ \mathrm{evolved} = 0.93.\log\ (NO_3^-) + 0.0231.(H_2O\ \%) \qquad (1)$$

Figure 6.5 shows the agreement between observed and calculated denitrification rates using the multiple regression model. In addition, the descriptors of both log-normal distributions (mean and standard deviations) were not significantly ($p<0.05$) different (Figure 6.6).

No significant regression was obtained for the production of N_2O in the samples without acetylene.

6.3 Laboratory investigations

A series of experiments were performed within the laboratory to further investigate the effects of environmental parameters on the denitrification rate in sediment of the Swale-Ouse system.

6.31 Temperature

During the seasonal investigation of denitrification along the Swale-Ouse, cores were incubated at ambient temperature at the time of sampling. However, on any particular sampling day, water and air temperature will vary according to the time of day. Thus, the aim of this study was to investigate the effect of temperature on denitrification rate on a seasonal basis within the possible ambient yearly temperature range.

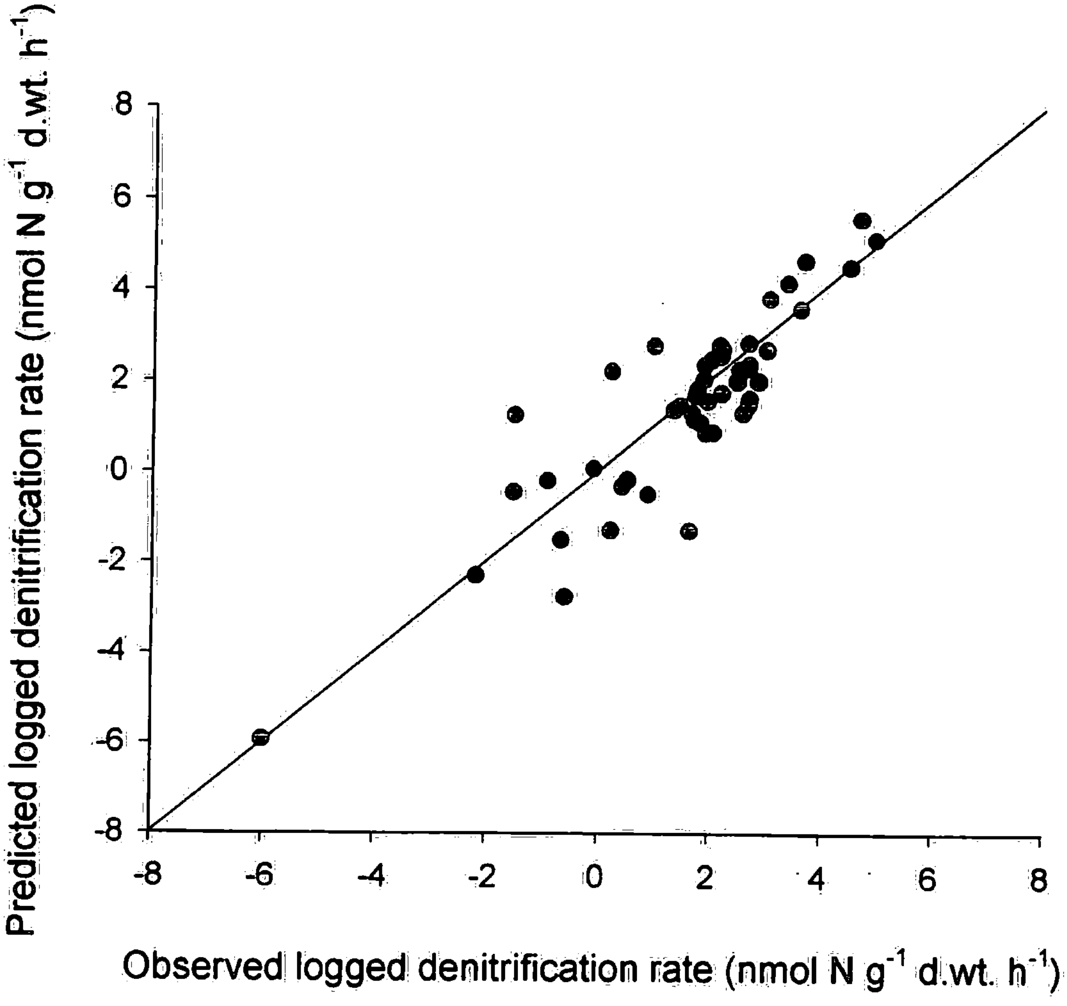


Figure 6.5 Measured, versus predicted, denitrification rates according to the multiple regression model for river nitrate and water content from the 50 river sites survey (September 1996).

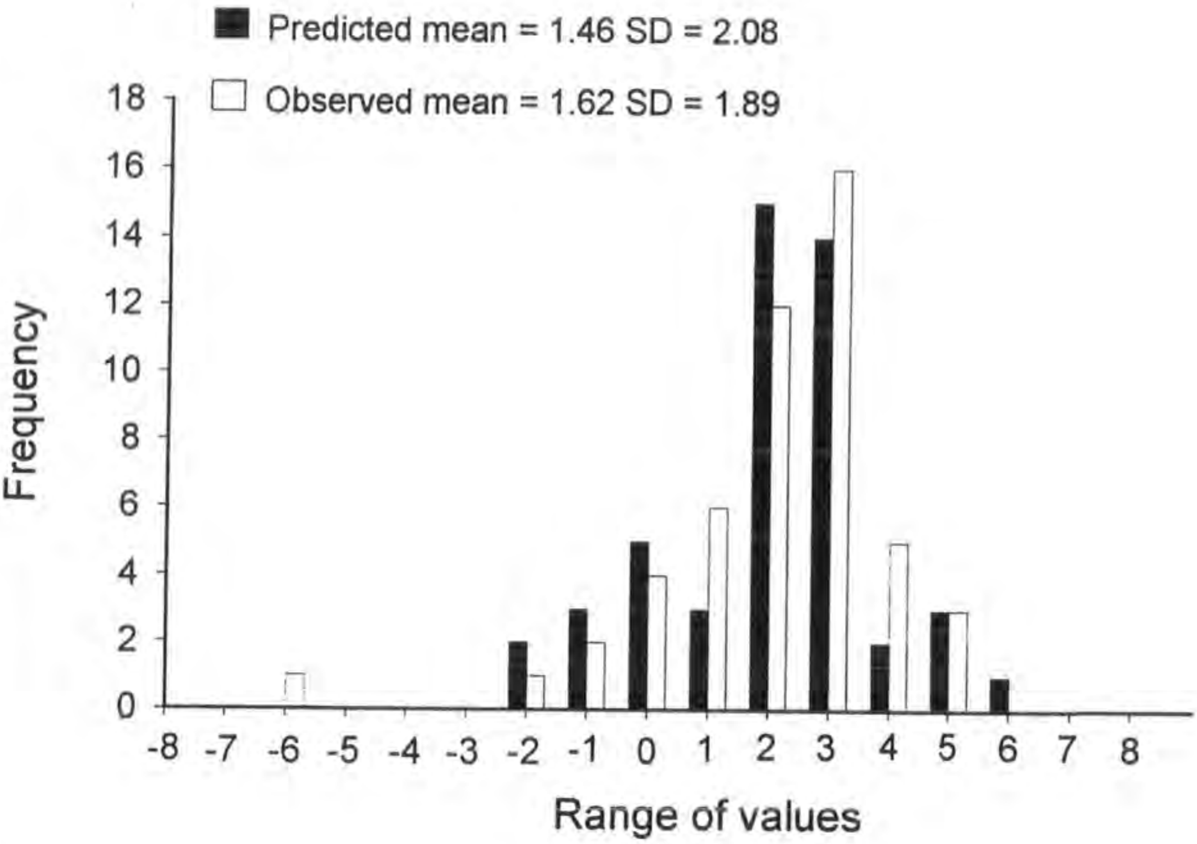
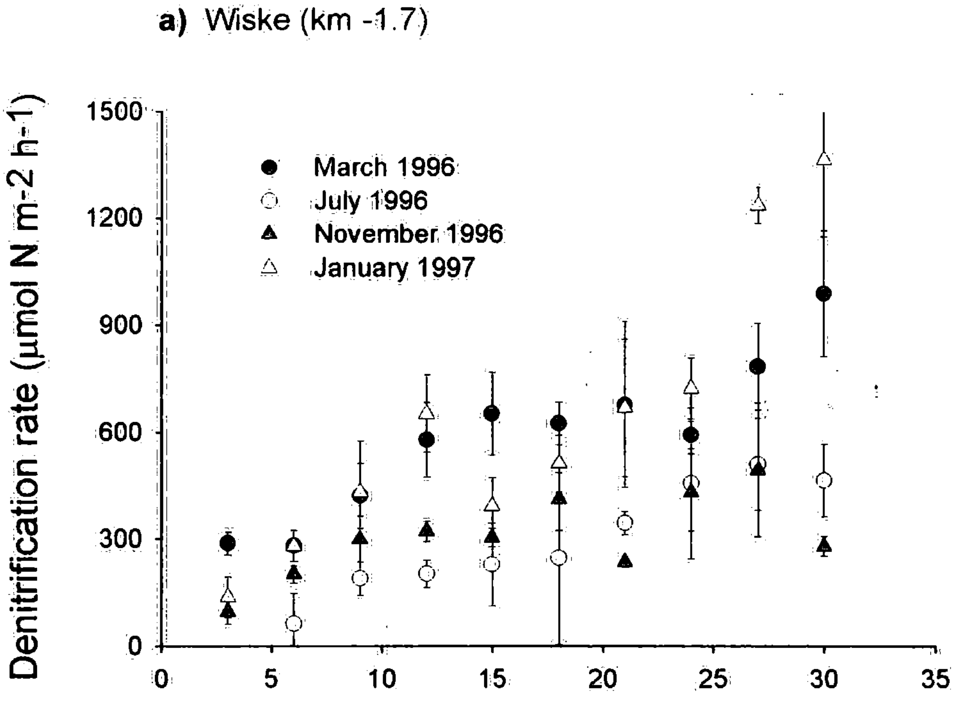


Figure 6.6 Frequency distribution of potential denitrification rates for (a) measured values and (b) predicted values from the 50 river sites survey (September 1996).

Comparative seasonal temperature curves were made at an upstream and a lowland site (Ivelet Bridge, km 10.9, and Wiske, km –1.7, 86.1). Denitrification rates were measured in intact cores from the Wiske, collected from the sites on four dates (18 March 1996, 2 July 1996, 1 November 1996,31 January 1997). For practical reasons Ivelet Bridge was sampled on different dates (10 July 1996, 30 October 1996, 5 February 1997, 8 April 997). Laboratory incubation was started within 3 h of collection and the assay commenced after 30 min of acclimation to the assay temperature. Denitrification rates for both sites were assayed at temperature intervals of 3°C between 3 and 30°C, thereby covering more than the range of water temperature recorded at the site. Three replicate cores were incubated with C_2H_2 at each temperature; in addition, three cores were used to determine the initial N_2O concentration. Cores were incubated for 4 h in the dark in a series of water baths.

Denitrification rate using intact sediment cores generally increased linearly with the temperature for the studies in the Wiske (Fig. 6.7a) and at Ivelet Bridge (Fig. 6.7b), although in January in the Wiske and in February at Ivelet Bridge the y-intercept values were negative suggesting a relationship resembling more that of an exponential curve. The slopes and intercepts were different on the different occasions. The slopes were higher in January and March than July for the Wiske (Table 6.6) and highest in February for the Swale (Table 6.6).



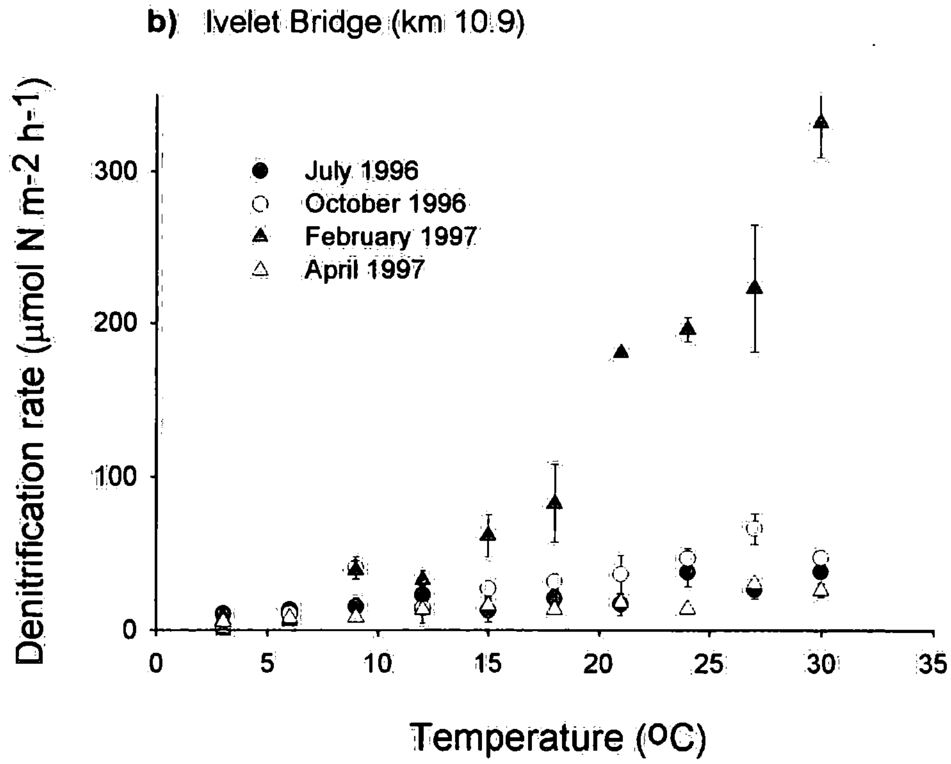


Figure 6.7 Effect of temperature on denitrification rate in intact sediment cores from the Wiske (a) and Ivelet Bridge (b). (Mean ± S.D.; n = 3).

Table 6.6 Relationship between denitrification rate and temperature in sediment cores (n = 3) on four dates in the rivers Wiske and Swale. Slope indicates the increase in denitrification rate per °C. y-intercept is the expected denitrification rate at 0° C; R^2 is the coefficient of determination for the relationship.

date	slope (μmol N m^{-2} h^{-1} °C^{-1})	y-intercept (μmol N m^{-2} h^{-1})	R^2
Wiske			
18 Mar 1996	22.1	224	0.85
2 Jul 1996	13.0	8.7	0.68
1 Nov 1996	-	-	-
31 Jan 1997	39.1	-5.1	0.81
Ivelet			
10 Jul 1996	0.89	6.64	0.67
30 Oct 1996	1.80	2.51	0.71
5 Feb 1997	11.6	-76.3	0.90
8 Apr 1997	0.76	2.72	0.78

6.32 Nitrate

6.321 Introduction

From the results of the present study and from previous investigations (see Section 1.21) nitrate is known to be a key environmental factor influencing denitrification rate. In addition to this, the present study has established that as nitrate increases on passing downstream, so does denitrification rate. However, there have apparently been no studies undertaken to determine the apparent affinity and capacity for nitrate utilisation of denitrifying bacteria down a whole river; although, this is important for clarifying the relationship between nitrate availability in the environment and nitrate use by denitrifying bacteria.

Previous attempts to measure the kinetics of nitrate utilisation in soils and sediments have been hampered by a number of problems. The earliest studies on nitrate utilisation employed slurry techniques (Oren & Blackburn, 1979; Hordijk *et al.*, 1987; Murray *et al.*, 1989), where the structure of the sediment is destroyed and hence the *in situ* oxygen and nitrate gradients. The measures of affinity and capacity for nitrate utilisation obtained with this method are only meaningful under conditions of no limitation, and cannot be incorporated into integrating models to predict biological features of lotic systems. Other researchers have used intact sediment cores to study the kinetics of nitrate reduction through measuring the decrease in nitrate concentration in the overlying water (Andersen, 1977). The long incubation period used, possible errors in measuring the nitrate concentrations, especially at low concentration, and the unknown fate of the depleted nitrate, make uncertain the significance of the reported kinetic parameters.

The aim of this study was to investigate the effect of increasing the nitrate concentration on denitrification rate and N_2O production in intact sediment cores taken from the five sites along the Swale-Ouse river and one on the Wiske. It should be noted that kinetic constants determined from this study reflect community activity (representing a mixture of enzymes) in a complex ecosystem, therefore they should be considered as "apparent". The term K_m (representing affinity) can only be applied to a pure enzyme, therefore, this study uses the term K_s to represent the apparent half saturation concentration or concentration of nitrate to obtain half of the apparent maximal velocity (V_{max}). Nevertheless, this method is still useful for characterising a group of enzymes for comparison between sites.

6.322 Additional methodology

Intact sediment cores were collected from the five Swale-Ouse sites and the Wiske site between 18 - 24 April 1997, with repeat sampling at two sites, upstream at Ivelet Bridge and the Wiske, on 18 June.

In order to establish the required nitrate concentration during experimental studies, it was necessary to remove nitrate and nitrite from the sediment. This was done using natural denitrification activity, as adapted from Murray *et al.* (1989), with river water being substituted by freshwater Chu medium lacking N or P (see Section 3.311). All samples were then incubated in the dark for 24 h at 15°C.

Following incubation to remove environmental nitrate and nitrite, the supernatant water was drained off and replaced with freshwater medium of the required nitrate concentration supplied as KNO_3. Nitrate concentrations used in the assays were modified to reflect the expected denitrification rate and ranged from 0 to: 140 μM for the upstream sites; 357 μM for downstream sites; 11,428 μM for the Wiske. Two triplicate sets of cores incubated at 15 °C were used for each nitrate concentration assayed at each site to measure both net N_2O production and denitrification. In addition, another two sets of three cores were used to determine denitrification and N_2O production using natural river water from each site.

The apparent kinetic parameters for denitrification were computed using a similar procedure to that used in Michaelis-Menten kinetics calculations by the Lineweaver-Burk transformation (1/*V* vs 1/S) of the Michaelis-Menten equation. "*V*" is the denitrification rate while "S" is the concentration of nitrate.

6.323 Results

Nitrate depletion. The denitrification rate and N_2O production in the cores following incubation for 24 h to remove sediment nitrate and nitrite are shown in Table 6.7. Denitrification was still detectable, though rates were low: the rates were 5 μmol N m^{-2} h^{-1} at Ravenseat and 31.4 μmol N m^{-2} h^{-1} in the Wiske. These values represent, respectively, 16 % and 3% of the maximum rates found during assays with the saturation level of nitrate (see below). N_2O production was very low in the Wiske (2.7 μmol N_2O-N m^{-2} h^{-1}) and lower or negative (net consumption of N_2O) at the other sites (Table 6.7).

Table 6.7 Net N_2O production (N_2O prod.) and denitrification rate (DR) in sediment cores (n = 3) from the Swale-Ouse system with no nitrate after 24 hours at 15 °C with N-free freshwater medium.

River	Site		Distance km	N_2O prod. ± SD μmol N_2O-N m^{-2} h^{-1}	DR ± SD μmol N m^{-2} h^{-1}
Swale					
	Ravenseat		-2.5, 0	0.74 ± 0.62	5.0 ± 4.0
	Ivelet Bridge	(April)	10.9	-1.42 ± 0.93	5.5 ± 0.6
		(June)		-2.34 ± 0.71	3.5 ± 0.7
	Catterick Bridge		49.9	0.25 ± 0.61	13.4 ± 1.6
	Thornton Manor		107.9	1.23 ± 0.56	12.2 ± 3.4
	Naburn Weir		145	0.83 ± 0.86	9.2 ± 0.36
Wiske					
	Wiske	(April)	-1.7, 86.1	2.72 ± 2.52	23.1 ± 9.4
		(June)		-1.57 ± 0.13	31.4 ± 3.8

Kinetics for denitrification and N_2O production. Nitrate addition in the overlying water resulted in an increase in denitrification rate (Fig. 6.8). The response of

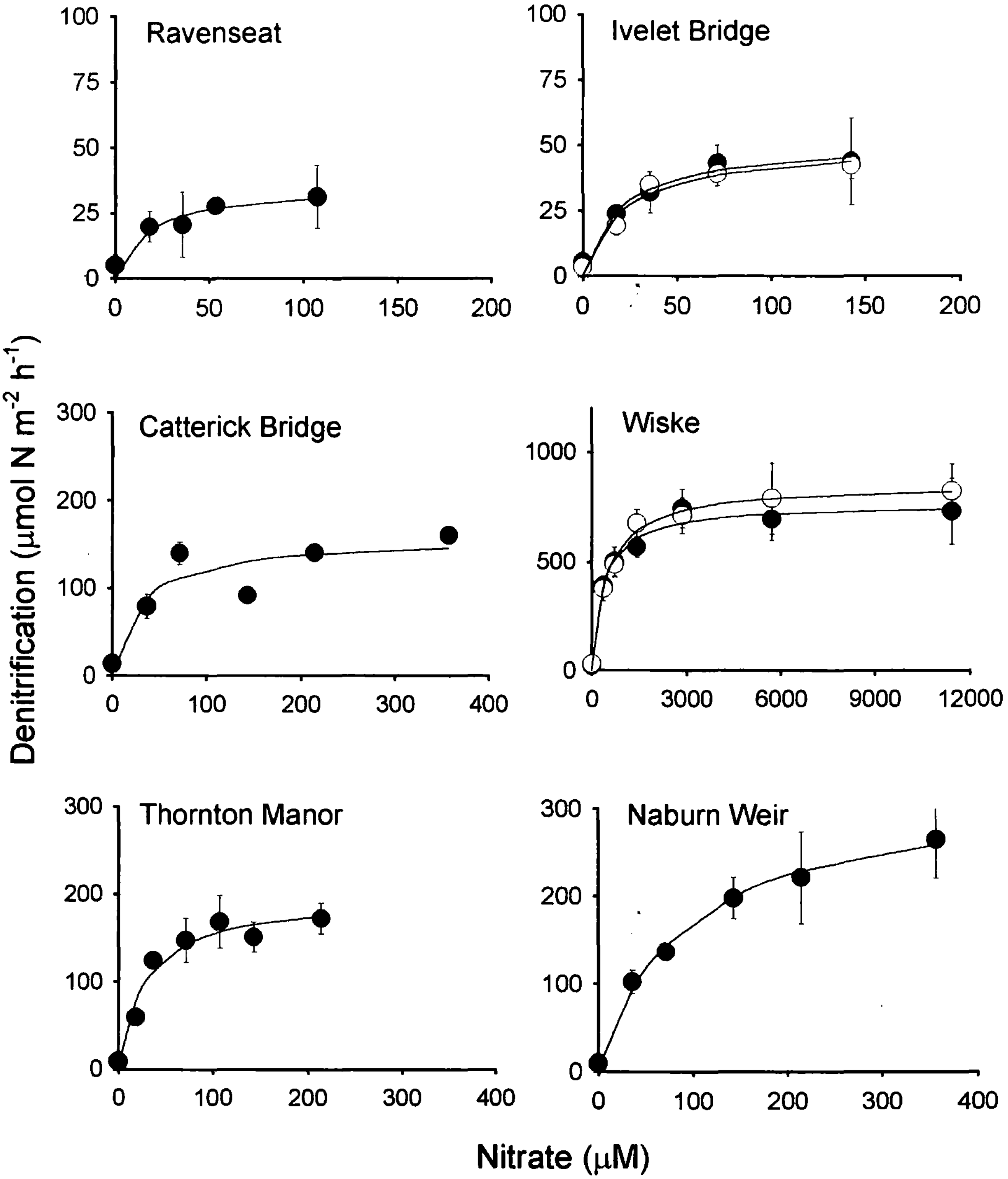


Figure 6.8 Response of denitrification rate to nitrate additions in sediment cores from the Swale-Ouse system in April 1997 (full circles). For Ivelet Bridge (km 10.9) and Wiske (km –1.7, 86.1) the assay was repeated in June 1997 (empty circles). Bars denote standard deviations of three replicates. Note the different scales for denitrification rate and nitrate concentrations.

denitrification to nitrate at all sites could be fitted successfully (minimum regression coefficient of 0.9) to a saturation type curve (Fig. 6.8 and Table 6.8), showing the apparent nitrate affinity (K_s) for mixed populations of denitrifying bacteria at each site. During the April survey of all sites (Table 6.8), apparent maximum activity (V_{max}) increased on passing down river from 35.8 μmol N m^{-2} h^{-1} in the headwaters at Ravenseat (km –2.5, 0) to 324.1 μmol N m^{-2} h^{-1} at the tidal limits at Naburn Weir (km 145.0), but was highest on the Wiske (758 μmol N m^{-2} h^{-1}). Apparent half-saturation (K_s) constants increased in April on passing down the main river from 13.1 μM nitrate at Ravenseat to 90.4 μM at Naburn Weir (Table 6.8). The overall highest value for K_s (460 μM) was for the Wiske in June. Where repeat measurements were made (Ivelet Bridge and Wiske), values for V_{max} and K_s were similar in April and June, Table 6.8.

Table 6.8 Kinetics parameters for denitrification rate from sediment cores (n = 3) from the Swale-Ouse system estimated according to calculations following a similar procedure to the Lineweaver-Burk transformation of the Michaelis-Menten equation.

River	Site		Distance km	V_{max} μmol N m^{-2} h^{-1}	K_s μM	R^{2a}
Swale			-2.5, 0			
	Ravenseat		10.9	35	13	0.95
	Ivelet Bridge (April)			52	21	0.98
		(June)		51	23	0.98
	Catterick Bridge		49.9	144	18	0.90
	Thornton Manor		107.9	197	29	0.98
	Naburn Weir		145	324	90	0.99
Wiske						
	Wiske	(April)	-1.7, 86.1	758	351	0.99
		(June)		860	460	0.99

[a] coefficient of determination

The production of N_2O in the cores without C_2H_2 could only be fitted to a saturation curve for three sites (Catterick Bridge, Naburn Weir and Wiske: Fig. 6.9). At the two upstream sites (Ravenseat and Ivelet Bridge), N_2O production was less than

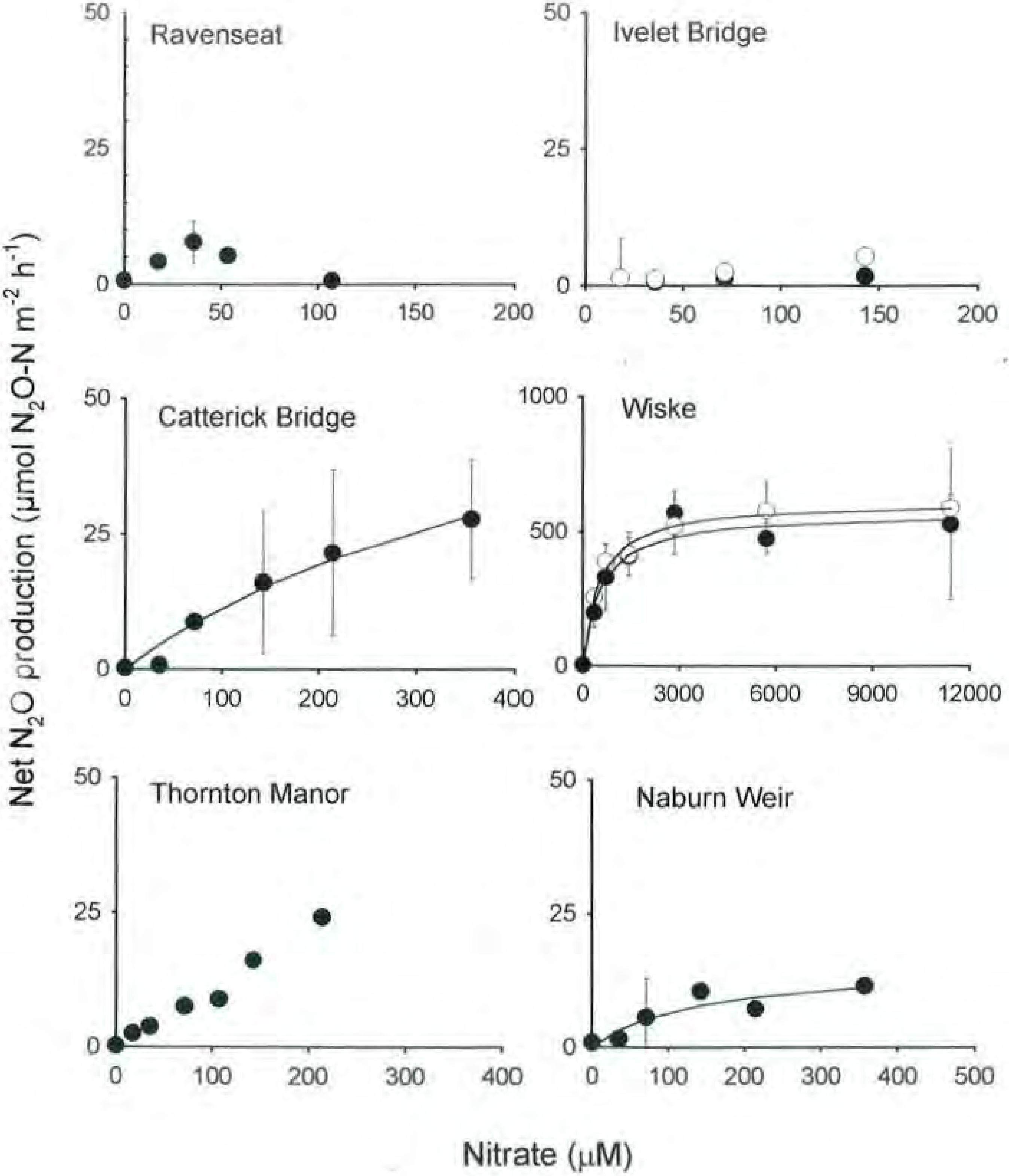


Figure 6.9 Dependence of the N_2O production upon nitrate in sediment cores from the Swale-Ouse system in April 1997 (full circles). For Ivelet Bridge (km 10.9) and Wiske (km -1.7, 86.1) the assay was repeated in June 1997 (empty circles). Bars denote standard deviations of three replicates. Note the different scales for N_2O production and nitrate concentrations.

15 μmol N_2O-N m^{-2} h^{-1} and without any clear trend with increasing nitrate concentration. Apparent maximum values for N_2O production were 70.2, 15.5 and 570 μmol N_2O-N m^{-2} h^{-1} in April for Catterick Bridge, Naburn and the Wiske, respectively, and 626 μmol N_2O-N m^{-2} h^{-1} in June for the Wiske (Table 6.9). K_s values were 517, 138 and 542 μM nitrate in April for the same sites and 532 μM nitrate in June for the Wiske (Table 6.9). The nitrate concentration to reach half of the apparent maximum velocity for N_2O production for these sites was higher than the K_s for denitrification rate, while V_{max} values were lower.

Table 6.9 Calculated kinetic parameters for N_2O production from sediment cores (n = 3) from the Swale-Ouse system estimated according to calculations following a similar procedure to the Lineweaver-Burk transformation of the Michaelis Menten equation.

River	Site		Distance km	V_{max} μmol N_2O-N m^{-2} h^{-1}	K_s μM	R^{2a}
Swale						
	Ravenseat		-2.5, 0	-	-	-
	Ivelet Bridge (April)		10.9	-	-	-
		(June)		-	-	-
	Catterick Bridge		49.9	70.2	517.4	0.98
	Thornton Manor		107.9	-	-	-
	Naburn Weir		145	15.5	138.6	0.93
Wiske						
	Wiske	(April)	-1.7,86.1	570.5	542.8	0.97
		(June)		626.6	532.1	0.98

[a] coefficient of determination.

The proportion of N_2O related to total N gases ($N_2O + N_2$) ranged between 2.4 to 38 % and 2 to 12 % at Ravenseat and Ivelet Bridge, respectively (Table 6.10), but without any clear trend with increasing nitrate concentration. The values ranged from 1 to 17 % at Catterick Bridge, 3 to 14 % at Thornton Manor (km 107.9) and 0 to 11 % at Naburn Weir (Table 6.10). The values increased on addition of nitrate at Catterick and Thornton Manor, but not Naburn Weir. In the Wiske values exceeded 50 % at all the nitrate concentrations in April and June (Table 6.10).

When river water (with its natural nitrate concentration) was used instead of medium, the denitrification rate was similar at all but one site to the rate predicted from the saturation curve for the nitrate concentration in the river, Fig. 6.10. At Ravenseat the denitrification rate was significantly (p<0.05) higher when using river water rather than medium. This suggests that differences between the artificial medium and the river water had in general a negligible effect on denitrification, at least during the incubation period of three to five hours. In the case of the headwater site, where the rate was higher, the most likely explanation is the presence of organic carbon substrate in natural stream water.

Table 6.10 Percentage of N_2O related to total N gases evolved (N_2O+N_2) from sediment cores (n = 3) in the Swale-Ouse system for the nitrate concentration assayed.

Nitrate (µM)	Ravenseat	Ivelet Bridge		Catterick Bridge	Thornton Manor	Naburn Weir	R. Wiske	
		April	June				April	June
17.8	21	6	7.5		4.3			
37.7	38	2	3.4	1.0	3.0	0.0		
53.6	19	3	6.4					
71.4				6.0	5.1	6.0		
107.1	2.3				5.3			
142.8		4	12	17	10	6.4		
214.3				15	14	3.2		
357.1				17		11	50	68
714.3							65	80
1428							71	62
2857							76	73
5714							68	73
11429							72	75

Ecological significance The K_s values calculated in this study for each site were compared (Fig. 6.11) with the nitrate data available for 1995-6 from other sources (Environment Agency and LOIS database). For the upland sites K_s values were higher than nitrate in the river, however, K_s values for all sites on the main river and the Wiske were lower than nitrate concentration for most of the year (except sometimes during summer).

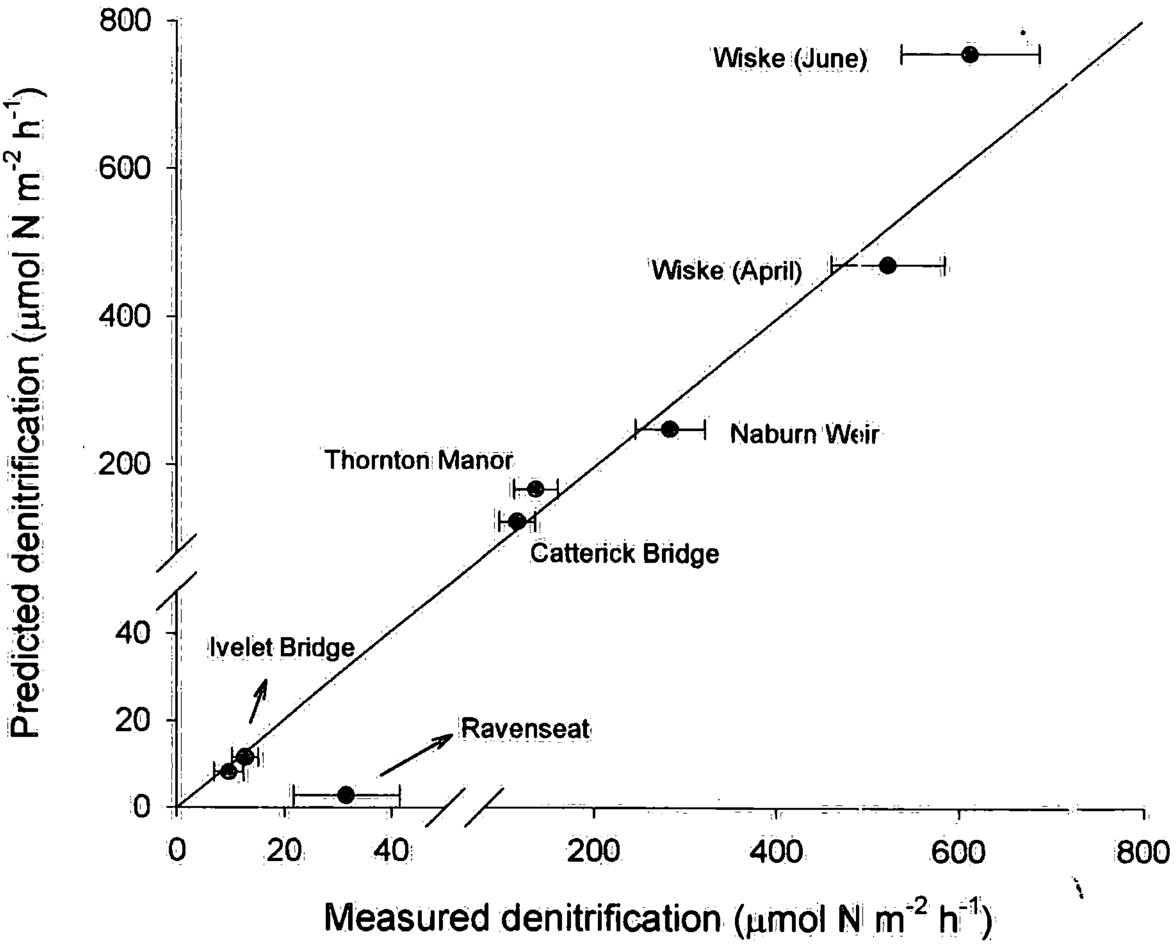


Figure 6.10 Comparison of denitrification rates in sediment cores from the Swale-Ouse system determined with natural river water, with those predicted according to K_s, V_{max}, and river nitrate concentration in April 1997 for all sites and in June 1997 for Ivelet Bridge and Wiske.

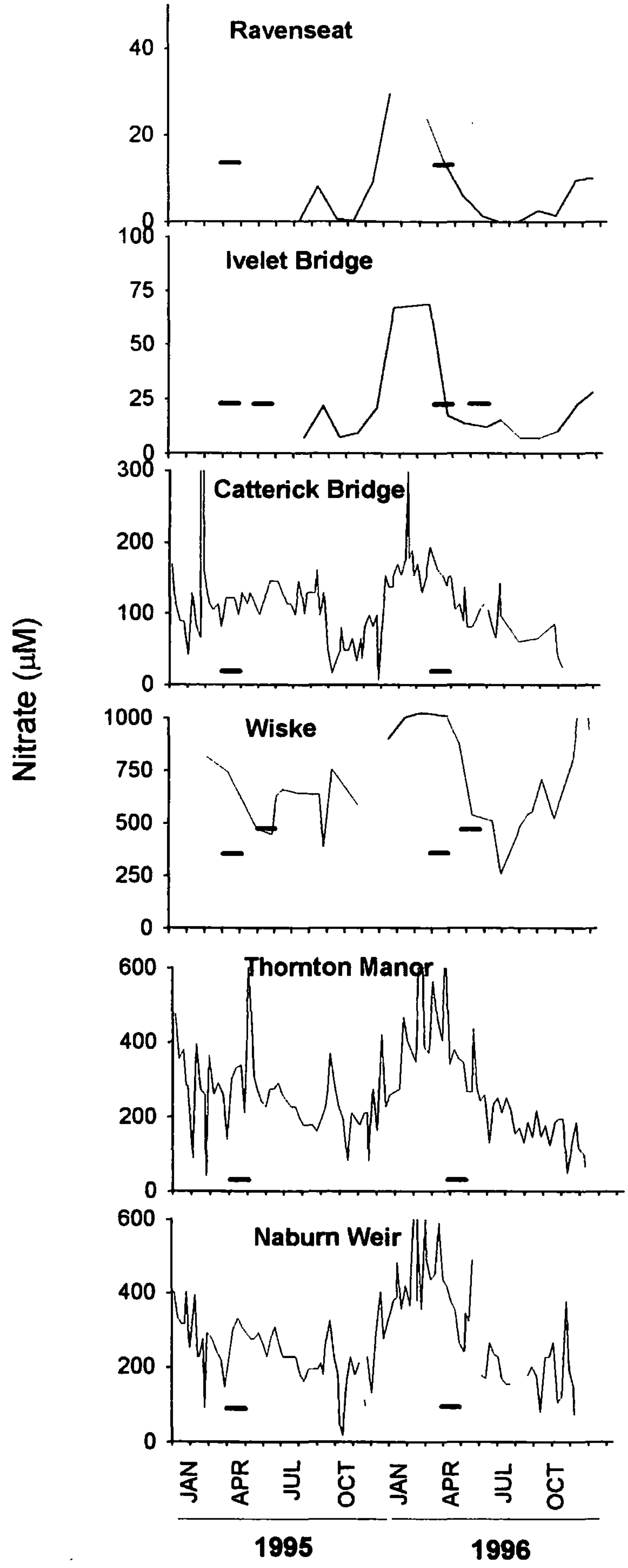


Figure 6.11 River nitrate concentration (Environment Agency & LOIS core data) for each sampling site on the Swale-Ouse system from January 1995 to December 1996. Horizontal lines show the concentration of nitrate to reach half of the apparent maximum velocity in April 1997 for all sites and June 1997 for Ivelet Bridge and the Wiske.

6.33 Nitrate and temperature

In the field study investigating the relationship of multiple environmental factors with denitrification at 50 sites in N-E England, temperature was not assessed as one of the multiple variables, this is because it did not vary notably over the three week study period. To investigate the combined effects of temperature and nitrate on denitrification, highlighted in the seasonal study on the Swale-Ouse (Section 4.7), a laboratory study using intact sediment cores taken from the Wiske (km 86.1, -1,7) and Ivelet Bridge (km 10.9) was undertaken on 5 and 14 March 1997 respectively. A series of triplicate cores were incubated at four different concentrations of nitrate (0, 714, 1428, 2143 and 0, 36, 72, 143 µM N as KNO_3 for the Wiske and Ivelet Bridge respectively) under three different temperature regimes (5, 10, 20°C). Before starting the assay, natural denitrification was used to deplete the *in situ* nitrate, as adapted from Murray *et al.* (1989).

The relationship between temperature and nitrate with denitrification at the Wiske and Ivelet Bridge is illustrated in Figs. 6.12 and 6.13. Denitrification increases in response to increases in temperature and nitrate at both sites. Regression analysis shows a highly significant relationship between denitrification and these two variables. The nitrate-temperature curve for Ivelet Bridge can be described by Equation 2:

$$(N_2+N_2O)\text{-N evolved} = 1.93 + 6.52.(NO_3^-) + 0.699.(\text{temperature}) \qquad (2)$$

The proportion of variation in denitrification rate that can be accounted for by each variable in this equation is 78.6 % ($p< 0.001$). Equation 3 describes the relationship between nitrate, temperature and denitrification in Wiske sediment.

$$(N_2+N_2O)\text{-N evolved} = -91.0 + 22.3.(NO_3^-) + 30.3.(\text{temperature}) \qquad (3)$$

This equation describes 91.4 % ($p< 0.001$) of the variation in the denitrification data. Additionally, nitrate describes a greater proportion of the variability than temperature at each site; 60 % and 63 % at Ivelet Bridge and the Wiske respectively.

6.34 Carbon

Organic carbon (C) is another factor known to potentially affect denitrification rate. The influence of carbon source on denitrification rate was investigated on

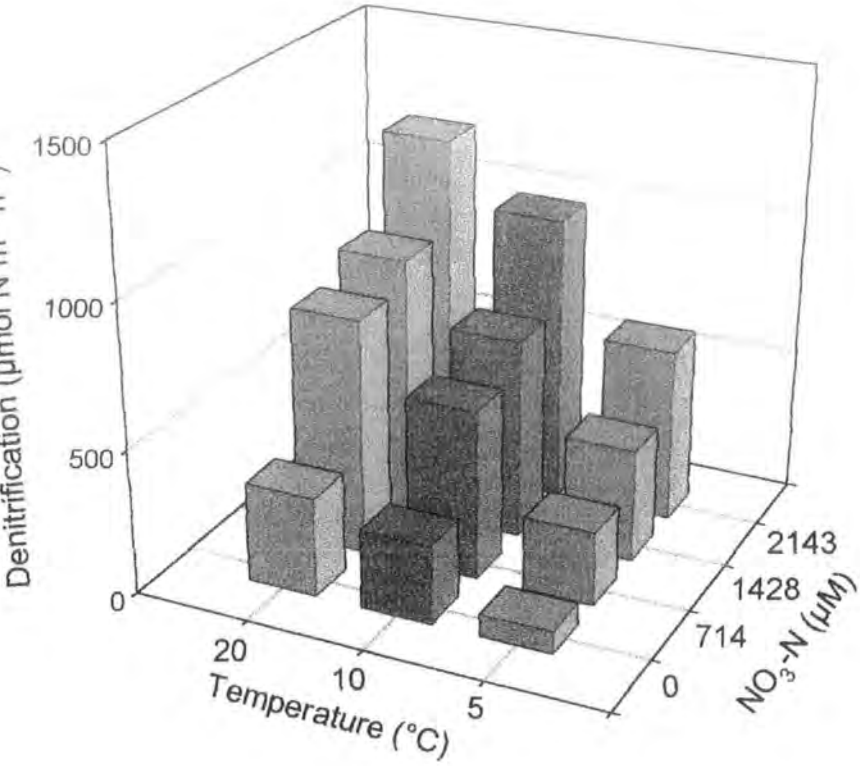


Fig 6.12 Influence of nitrate and temperature on denitrification in sediment cores from the River Wiske (5/3/97).

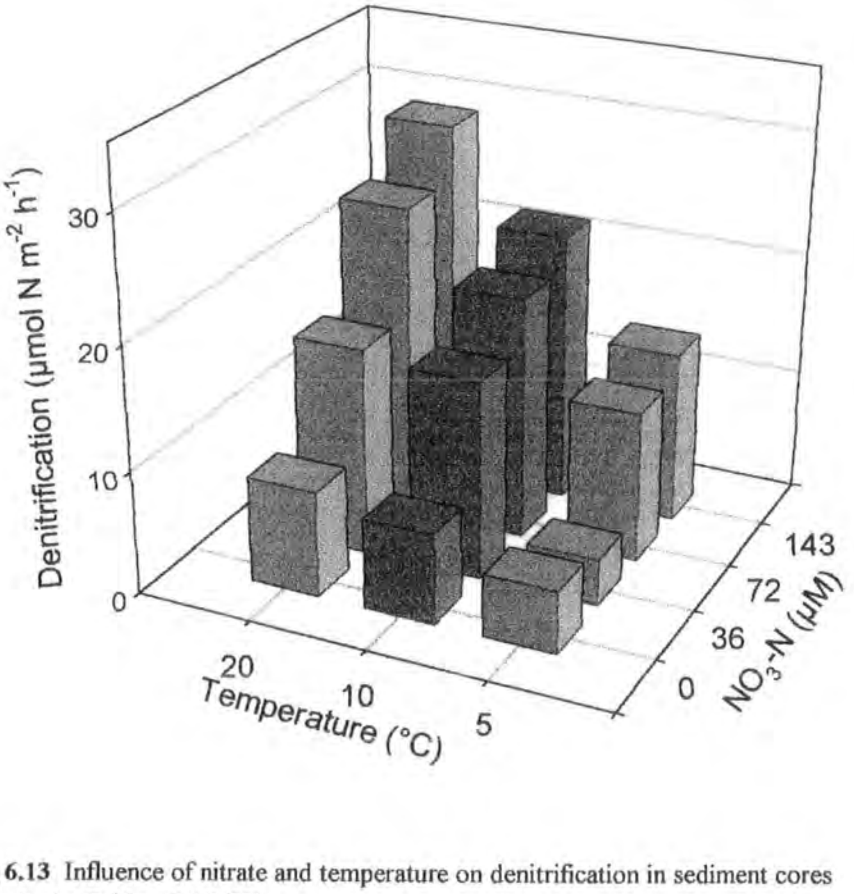


Fig 6.13 Influence of nitrate and temperature on denitrification in sediment cores from Ivelet Bridge (14/3/97).

15 November 1996 using the slurry technique to measure potential denitrification in the top 5 cm. Jars were filled with dilute inorganic medium (modified version of No. 10 medium of Chu, 1942) enriched with 20 mg l^{-1} NO_3-N (as KNO_3) and 100 mg l^{-1} C of the organic carbon source under test. The carbon sources tested were methanol, glucose, ethanol and sodium acetate, together with a control lacking organic carbon. Jars were sealed, without any head space and incubated statically for 4 h in the dark at 15°C. Following incubation and gas analysis, potential denitrification was calculated as described in Section 3.311.

In spite of the high concentration of carbon sources tested, none led to a significant ($p<0.05$) increase in denitrification rate (Table 6.11 and Fig. 6.14) compared with the control. However, the production of N_2O in the control was significantly higher ($p<0.05$) than in the C treatments and accounted for 22 % N gas produced, compared with the maximum of 4 % (for sodium acetate) when C was added (Table 6.11).

Table 6.11 Influence of various organic carbon sources (100 mg L^{-1} C of methanol, glucose, ethanol or sodium acetate) on potential denitrification rate, N_2O production and composition of N-gases (mean ± SD; n = 3) from river Wiske sediments (15/11/96).

Carbon source	Denitrification (μmol N m^{-2} h^{-1})	N_2O production (μmol N_2O-N m^{-2} h^{-1})	Composition of the N gases produced (%)	
			N_2	N_2O
Glucose	258 (15.9)	4.4 (1.5)	98.3	1.7
Methanol	237 (26.4)	7.9 (2.4)	96.7	3.3
Ethanol	233 (11.6)	5.9 (0.4)	97.5	2.5
Acetate	245 (26.1)	10.6 (1.6)	95.7	4.3
Control	299 (50.4)	67.5 (2.9)	77.4	22.5

6.35 Oxygen

A limited investigation into the possible effects of O_2 concentration on denitrification was performed on sediment collected from Naburn Weir, Cawood and Selby on 17 July 1997. This was done by comparing potential denitrification assays in river water which had been bubbled for 5 min with air versus ones with water which had been bubbled for 5 min with N_2. The jars were sealed without any gas space and

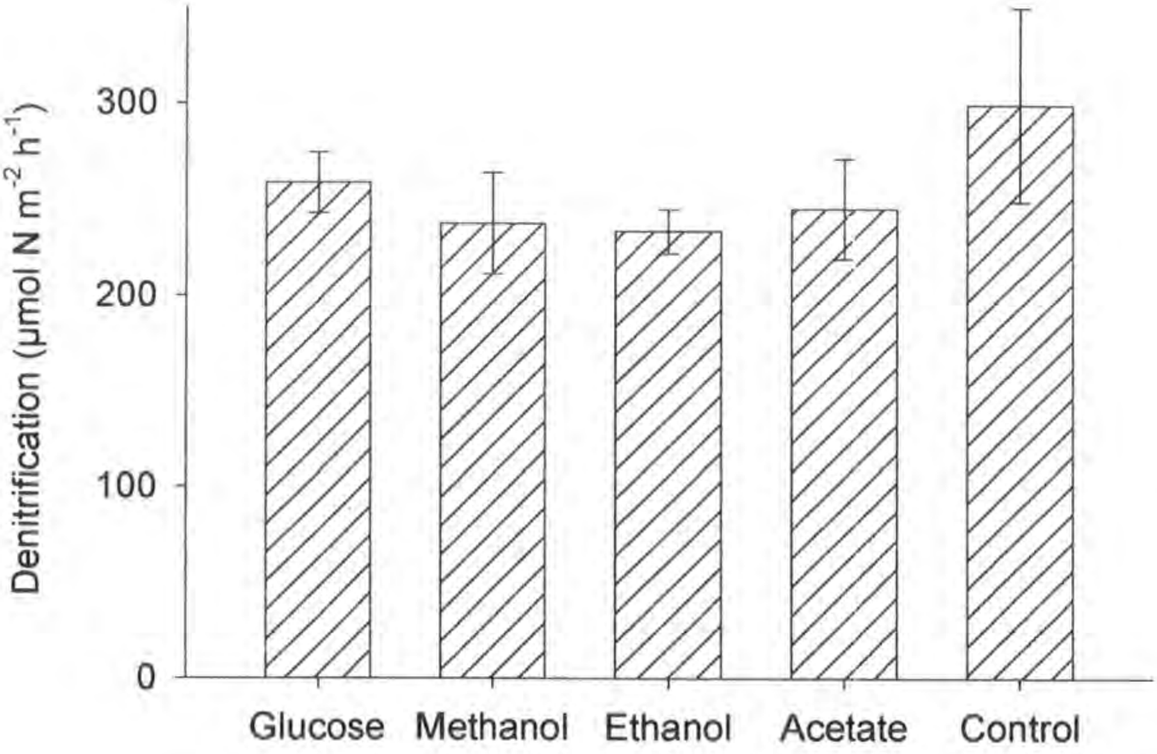


Figure 6.14 Influence of various organic carbon sources (100 mg L^{-1} C of glucose, methanol, ethanol and sodium acetate) on denitrification rate in Wiske sediment (15/11/96). (Mean ± S.D.; n = 3)

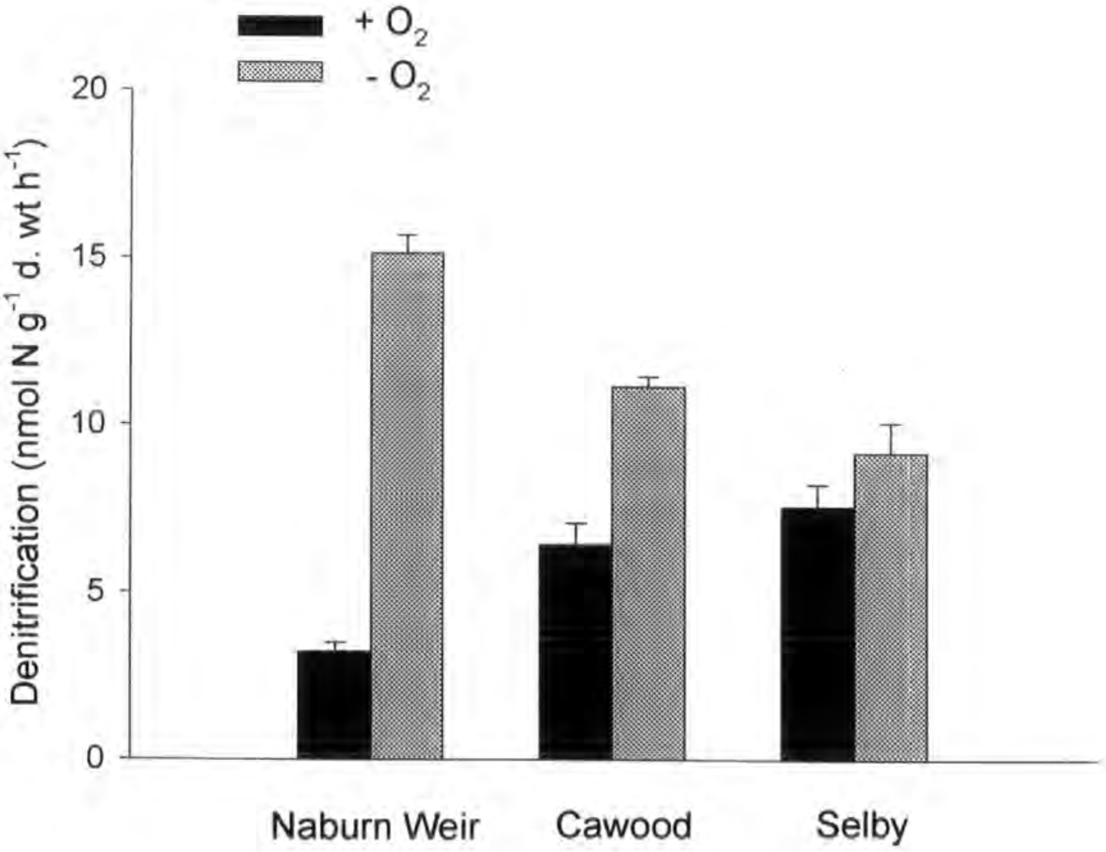


Figure 6.15 Influence of oxygen status on potential denitrification rate in sediment from the freshwater tidal Ouse (17/7/97). (Mean ± S.D.; n = 3). $+O_2$ and $-O_2$ indicate whether or not the overlying water was gassed with N_2 prior to the experiment.

incubated statically for 4 h in the dark at 15°C. The standard procedure was used for analysis of N_2O.

Purging the water with N_2 to reduce the O_2 concentration had no effect on denitrification at Cawood and Selby, although the rate at Naburn Weir was slightly higher in jars with reduced O_2 (Fig. 6.15).

6.4 Discussion

Data presented in this chapter clarifies the relationship between denitrification rate and the chemical and physical characteristics of water and sediments through both intensive field and laboratory investigations. This means that the hypothesis that certain environmental factors exert a greater influence on denitrification than others can be confirmed (Section 1.4).

The studies in the Swale-Ouse and Tweed had already highlighted some key influencing factors on denitrification, such as nitrate concentration, sediment and temperature, however, this was just within one system. To substantiate the hypothesis posed, it was therefore considered necessary to clarify the relationships between rates of denitrification and N_2O production and environmental factors likely to influence these processes. To achieve this it was decided to investigate a large number of rivers, chosen to represent a wide range of conditions. Furthermore, to ensure that statistical comparisons were valid, it was attempted as far as possible to sample under one set of climatic conditions. For these reasons the survey of denitrification and N_2O production in river sediments at 50 sites in N-E. England in late summer was undertaken.

With the exception of the headwater site in the Swale catchment (Ravenseat), denitrification was detected at all sites; ranging from < 0.005 to 260 nmol N g^{-1} d. wt h^{-1}. The absence of detectable denitrification at Ravenseat was probably due to the low concentration of aqueous nitrate (0.24 μM) and sediment total C (91 μg g^{-1} d. wt), making it difficult to support the process. The highest denitrification rates were always found in the organically polluted lowland rivers, such as the River Wiske, where sediments tended to be covered by decomposing algal or other organic debris.

Where more than one sample was taken within a particular river system (e.g. Tyne, Wear, Tees and Swale) denitrification rate tended to increase on passing downstream. A combination of environmental factors appear to explain this trend. There were strong interrelationships evident within the environmental variables measured; for example, sediment water content was correlated with C and N content, as well as fine particles of sediment. However, the key influencing environmental variables are probably aqueous nitrate and sediment variables, since denitrification correlated positively with nitrate concentration of the water column, water content of the sediments and percentage of fine (< 100 μm) particles in the sediment. This may be due to the fact that as sediments become finer, so there is a greater potential for anoxic environments and microbial attachment. Furthermore, there is an increase in contents of C and N; all of these factors would result in more favourable conditions for populations of denitrifying bacteria.

The multiple regression analysis established that the variability of denitrification rates could be satisfactorily explained (77%) by the full range of sediment and water characteristics from this study. Mean estimates of rate obtained from the multiple regression model calculated from the data acquired from the 50 sites did not differ significantly from the measured rates (see Fig. 6.5). In addition, the model accurately described the variability (shape of the histograms, see Fig. 6.6) exhibited by natural denitrification rate estimates. As suggested above, aqueous nitrate and sediment water content explained the greatest amount of variation (64 %) in the denitrification data between them; aqueous nitrate in fact explained the most (53 %). This would suggest that nitrate is the key influencing factor on denitrification within these river sites. As discussed above, sediment water content was highly correlated with sediment percentage of fine particles, C and N contents, so it may be for this reason that sediment water content explains so much of the variation in denitrification data.

Nitrous oxide production ranged from negative values (net consumption) to 13 nmol N_2O-N g^{-1} d. wt h^{-1} and accounted for 0 to 115% of the N gases produced. Similarly to denitrification rate, N_2O concentrations in the sediment correlated positively with nitrate concentration of the water column, water content of the sediments and percentage of fine (< 100 μm) particles in the sediment. However, following statistical analyses, no simple or multiple relationship was found for N_2O production. This is possibly because N_2O accumulation depends not only on its synthesis, but also on the extent of its reduction to N_2 by reductase enzymes (Section 1.2), which could therefore result in a complex combination of influencing factors.

The comparative seasonal temperature curves were made on intact sediment cores from the Wiske and Ivelet Bridge (Section 6.31). Denitrification generally increased linearly with temperature showing a clear response to a rise in temperature, however, the slopes and intercepts were different on the different occasions. This could be as a consequence of denitrifying bacteria populations varying with season. A further laboratory study investigated the combined effects of temperature and nitrate on denitrification using intact sediment cores from the Wiske and Ivelet Bridge (Section 6.33). This showed a highly significant relationship between denitrification and both variables ($R^2 = 0.91$, $p<0.001$ and $R^2 = 0.79$, $p<0.001$ for the Wiske and Ivelet Bridge respectively), where an increase in both temperature and nitrate results in an increase in denitrification. This experiment also shows that nitrate affects denitrification at both sites, furthermore from the different scales (Figs. 6.12 & 6.13) it can be seen that the affinity for nitrate at both sites is very different.

Having established from the previous studies the fact that nitrate is a key influencing factor on denitrification, it is possible that affinity for nitrate of denitrifying bacteria may vary depending on their environment. Therefore, it was decided to clarify the relationship between nitrate availability in the environment and nitrate use by denitrifying bacteria. In order to achieve this, kinetic parameters for nitrate reduction were calculated along the Swale-Ouse river system by using the acetylene blockage method with intact sediment cores (Section 6.32).

The denitrification rate in sediment containing added nitrate exhibited a saturation type curve (Michaelis-Menten) which meant that the mixed denitrifying bacterial populations within the sediment were responding to the nitrate additions. Furthermore, repeat measurements on different dates at Ravenseat and on the Wiske showed only moderate differences in curves (Figure 6.8). Estimates of apparent maximum velocity (V_{max}) ranged from between 35.8 and 324 μmol N m^{-2} h^{-1} in the Swale-Ouse (increasing upstream to downstream) and was highest in the Wiske (1194 μmol N m^{-2} h^{-1}). This can be related to the increase in nitrate with distance from the source, however, it is also possible that other environmental factors may also influence the bacterial populations. These factors could include for example, numbers of denitrifying bacteria, particle size of sediment and available organic C.

Apparent affinity (K_s) for nitrate by the mixed populations of denitrifying bacteria increased on passing downstream (high K_s to low K_s) from 13.1 to 90.4 μM in the main river, but was highest (640 μM) in the Wiske, therefore mirroring the trend in nitrate concentrations. This would suggest that the upstream mixed bacterial populations, where substrate (nitrate) is low, can actually utilise the substrate more efficiently, therefore showing a greater affinity for nitrate than bacterial populations further downstream where there are greater concentrations of nitrate. When the K_s values calculated in this study for each site were compared with the nitrate data available for 1995-6 from other sources (see Fig. 6.11) it became apparent that the K_s values generally fitted into the range of nitrate concentrations recorded for each site. This would suggest that the mixed populations of denitrifiers at each site are well adapted to the usual levels of nitrate.

The study of the kinetics of nitrous oxide (N_2O) production showed that although net N_2O production occurred at all sites, there was not always an increase with increasing nitrate concentration at all sites (see Fig. 6.9). It is difficult to explain this since N_2O is an intermediate product of at least three processes within the nitrogen cycle (Section 1.3). However, it is possible that once again nitrate is a key influencing factor since rates ranged from below the detection limit (0.05 μmol N_2O-N m^{-2} h^{-1}) at Ravenseat (with lowest nitrate concentration) and 15.5 μmol N_2O-N m^{-2} h^{-1} at Naburn Weir (with highest nitrate concentration in the main river). In the Wiske (with the overall highest nitrate concentration), the rate was up to 570 μmol N_2O-N m^{-2} h^{-1}, and accounting for up to 76 % of total N gas production.

Addition of organic substrates had no detectable effect on denitrification rate in intact sediment cores from the Wiske, although it did lead to a significant decrease in the proportion of nitrous oxide produced as a result of nitrate reduction (Section 6.34). This might suggest that C was not a limiting factor for denitrification at this particular site, at least in November when the investigation was performed. The added C would, however, also be expected to affect respiration of the microbial population as a whole and thus reduce the ambient oxygen which may be the reason for a decrease in percentage N_2O with C addition.

Finally, there was little or no effect on the potential rate of denitrification observed when sediment slurries from the freshwater tidal reaches of the Ouse were incubated with reduced oxygen (Section 6.35). This may be indicative of the fact that the general instability of the sediments in the tidal reaches of the Ouse may have had a greater influence on the denitrifying bacterial populations than the lack of oxygen. This is potentially confirmed by the fact that there is a greater increase in potential denitrification with no oxygen at Naburn Weir at the tidal limits than at Cawood or Selby.

This discussion has provided a review of the key findings of the field and laboratory investigations intended to clarify the relationship between denitrification rate and the chemical and physical characteristics of water and sediments; these findings are further discussed in Chapter 7.

CHAPTER 7

DISCUSSION

7.1 Introduction

In order to assess the trends and influences of denitrification in sediments in river systems, as laid out in the original hypotheses and consequent objectives (Section 1.4), this chapter will first consider the seasonal and spatial variation evident in the denitrification, physical and chemical data (Section 7.2) and then discuss the extent by which it is regulated by environmental factors (Section 7.3), before finally assessing the potential significance of denitrification in the removal of nitrate from the upper reaches of the Swale-Ouse (Section 7.4).

7.2 Seasonal and spatial trends

Denitrification

Denitrification rates measured in Swale-Ouse, Wiske and Tweed sediments were mostly of a similar order of magnitude to those reported for other rivers in temperate climates. For example, Torre *et al.* (1992) found an annual mean of 458 μmol N m^{-2} h^{-1} in the R. Charente, west central France using an *in situ* chamber to measure gas production. Rates from 364 to 2121 μmol N m^{-2} h^{-1} were found for an agricultural stream basin in New Zealand (Cooper & Cooke, 1984) and 41 to 375 μmol N m^{-2} h^{-1} in a lowland stream in Denmark (Christensen & Sørensen, 1988), both using acetylene inhibition.

Swale-Ouse system

Denitrification, like many other river processes (Vannote *et al.*, 1980), showed a gradient of change on passing downstream, with rates typically increasing by about two orders of magnitude from the headwater site (Ravenseat) to the tidal limit (Naburn Weir). As mentioned in the Introduction (Section 1.21), denitrification may be influenced by a variety of factors, such as nitrate, reducible organic substances, oxygen concentration and temperature. The increasing rate of denitrification on passing downstream is matched by increasing aquatic nitrate concentrations resulting from increased urban and agricultural activities in the lowland reaches (House *et al.*, 1997), with the concentrations at upper and lower limits typically differing by rather less than one order of magnitude. The fact that nitrate in the sediment did not match that in water by increasing to such a great extent may reflect the utilisation of nitrate by denitrification.

Sediment type also varies from upstream to downstream. For example, at the headwater site, there was a high percentage of coarse sand and gravel, and a low percentage of total carbon and hence probably low amounts of reducible organic substances. On passing downstream there was a consistent increase in percentages of silt and total carbon. The differences in the sediments may also be expected to lead to differences in denitrification due to the increased surface area available for microbial attachment on passing downstream. The survey of river sediment to enumerate denitrifying bacteria (Most Probable Number; Tiedje, 1982) in the top 5 cm showed values ranging from 2.4 x 10 to 9.0 x 10^5 g d. wt^{-1} on passing downstream (see Table 4.4). However, caution is needed in interpreting these values because of the uncertainty of the suitability of the method for sediments of widely differing types.

Although no data on oxygen concentrations in the sediments are available, decreased porewater flushing in fine sediments will result in less exposure to oxygen than in the coarser sediments upstream (Thompson *et al.*, 1995) and hence presumably favour denitrification. The water temperature difference between the upstream and downstream seldom exceeded 3°C and is therefore unlikely to be an important factor influencing denitrification rates down the river on any one day.

Despite the unfavourable conditions (low nitrate, probably low reducible carbon substrates, saturated oxygen concentration in the overlying water) for denitrifying bacteria at the headwater site, denitrification was still detectable (see Fig. 4.8). This suggests that denitrifying bacteria are more versatile than previously thought and that aerobic denitrification and/or microaerobic sites under aerobic conditions exist (Lloyd, 1993).

The seasonal bias towards a spring (April – May) peak in the denitrification rate has been observed in other riverine sediments (Christensen & Sørensen, 1988; Sørensen & Revsbech, 1990; Pinay *et al.*, 1993). The laboratory study showing the importance of nitrate concentration and temperature on denitrification in sediment from Ivelet Bridge and the Wiske (see Section 6.33) suggests that this peak may be due to the varying importance of nitrate and temperature at different times of year. Despite the high nitrate availability in December to February, denitrification may be expected to be limited by low temperatures. During spring, denitrification is increasingly favoured by rapidly rising temperatures whilst nitrate availability is still relatively high, but towards the end of this period nitrate concentration starts to decrease, perhaps due to decreased run off and increased macrophytic uptake (Howard-Williams *et al.*, 1982; Cooper, 1990). Nitrate concentration typically remains low until early autumn. In summer, denitrification is therefore probably nitrate-limited for much of the time.

In spite of the strong positive relationship ($R^2 = 0.91$) between nitrate, temperature and denitrification found in the laboratory study (see Section 6.33), the relationship was not so evident for any particular site when using field nitrate and temperature to predict denitrification (see Section 4.3). This could be due to the combined influence of other environmental variables throughout the year, resulting in the lack of significant correlations with denitrification. The kinetic studies (see Section 6.32) also support the strong relationship of denitrification with nitrate availability from overlying water within the Swale-Ouse system, these will be discussed below.

Instability in the upper sediment leading to decreased denitrification may be an important factor under conditions of high flow that could enhance or complicate the seasonal trend and regulation of denitrification. Unfortunately, the difficulty of sampling under such conditions means that relevant data were not obtained. High flows in winter and early spring can result in a great deal of sediment erosion and downstream transportation. This could potentially remove carbon from the surface layers (Jansson *et al.*, 1994), as well as leading to sediment instability, preventing the development of microbial communities and perhaps a greater penetration of dissolved oxygen. Such factors could also explain the decrease in denitrification rate from Cawood downstream to Selby in the freshwater tidal reaches.

The freshwater tidal part of the Ouse is a highly complex system and difficult to sample. In addition to the frequent changes in flow and direction of water movement, the sediment contains a relatively high component of fine particles, whose upper layer is continually being resuspended and redistributed, thus limiting the stability of the sediment and the formation of stable gradients in nutrients and processes. The decrease in denitrification at Selby is in striking contrast to all other measurements from the headwaters at Ravenseat to Cawood, where there was a progressive increase in denitrification rate at each successive site downstream (see Fig. 4.16). Nevertheless, the sediments are apparently responsible for the denitrification occurring at this site, as there was no evidence of denitrification in the water column in July under conditions which might have been expected to be optimal for denitrification to occur. The water column study was made at a time of year when oxygen concentrations may be expected to be at their lowest and temperature highest. The value of 24 % dissolved O_2 recorded for the day of the study in the water column is similar to the average values (25-30 %) of saturation recorded for this site in summer by Gameson (1982a). In addition, the study was carried out at low tide, when suspended particle concentrations tend to be highest (Gameson, 1982b). In general, the rate of denitrification in sediments of the freshwater tidal part of the Swale-Ouse is quite similar to that in the lower part of the non-tidal part of the river. However, the rates are much more variable at any particular site due to the instability of the sediments resulting in the lack of clear seasonal trends in this reach.

Nitrous oxide (N_2O) production

Nitrous oxide production in the Swale-Ouse showed obvious spatial changes and slightly less obvious seasonal changes (see Section 4.4). Similarly to denitrification, it seems probable that the increasing nitrate concentration in the water on passing downstream is an important factor influencing the tendency for N_2O production to also increase. Higher temperatures in summer are probably responsible for the general seasonal trend toward higher N_2O production then.

The increase in N_2O production with increasing nitrate on passing down river on individual days would fit with experimental studies on marine sediments, such as those of Law *et al.* (1991) who found a proportional increase in N_2O production with increased nitrate in the overlying water (see Section 1.3). However, other factors such as the dissolved oxygen and organic contents of the sediment may also have important influences on N_2O production and it would require experimental studies to determine their contributions, since the concentrations of nitrate in the water column and these three sediment variables are markedly inter-correlated. Aqueous phosphate also increases on passing downstream. Aquatic mosses growing in upstream tributaries of the Swale and the uppermost main river site (km 10.9) show evidence of phosphorus limitation (Christmas & Whitton, 1998a, b), so the possible influence of sediment phosphate at some sites needs to be considered. Differences between bacterial communities between sites may be another factor influencing N_2O production. A wide variety of bacteria are known to carry out denitrification (see Section 1.2) and N_2O can sometimes be the terminal product instead of N_2 (e.g. some pseudomonads, Greenberg & Becker, 1977).

The response of N_2O production to increase in nitrate and the significant relationship between N_2O production and denitrification suggests that denitrification is the process responsible for most of the observed production of N_2O. However, the effect of nitrate concentration on N_2O production is probably not a simple substrate-product relationship, since high nitrate concentration is known to partially inhibit nitrous oxide reductase in soils (Blackmer & Bremner, 1978; Letey *et al.*, 1981) and sediments (Terry & Tate, 1980) with a subsequent increase in the N_2O pool. The fact that N_2O production shows greater variability than denitrification at any particular site (see Table 4.8) suggests that the relative effect of environmental factors influencing N_2O removal may differ from that on the initial steps in denitrification (i.e. $NO_3^- \Rightarrow N_2O$). In addition to nitrate, it seems likely that small differences in oxygen or dissolved organic matter concentration may be especially important in the N_2O reduction step. However, it must be noted that care is needed when considering the production of N_2O: while total N_2O produced in the presence of C_2H_2 may be a good indicator of total denitrification ($N_2 + N_2O$), the production of N_2O in the absence of C_2H_2 may not be due solely to denitrification, but may also result from nitrification and dissimilatory nitrate reduction to ammonium (see Section 1.3).

Tweed

Data obtained from the Tweed support the seasonal and spatial trends found in the Swale-Ouse system. Although only four seasonal measurements of denitrification were performed at each site, they still serve to verify the spring peak in denitrification that was observed in the Swale-Ouse system, which would again potentially relate to increasing temperature and availability of nitrate. As with the Swale-Ouse, a spring/summer maximum in N_2O production was also found in this system.

Like the Swale-Ouse, spatial trends in various factors were also evident on the Tweed. The rates of denitrification measured in the Tweed were of a similar order of magnitude to those measured in the middle reaches of the Swale, which also had similar levels of nitrate. Once again the levels of denitrification activity mirrored the levels of nitrate on moving downstream, except for the freshwater tidal site at East Ord where there was a decrease in rate, similarly to Selby on the Ouse. This would again suggest the strong relationship between denitrification and environmental nitrate availability, except where other environmental factors such as sediment stability exerted an influence.

From the long-term field studies on both the Swale-Ouse and Tweed river systems, the hypothesis that a gradient of physical and chemical variables along a river contnuum can elicit a graded response from denitrifying bacterial populations

(Section 1.4) can be confirmed. Furthermore, the hypothesis that these physical and chemical variables will vary with season and will, therefore, elicit a seasonal response from the denitrifying bacterial populations can also be confirmed.

7.3 Factors affecting denitrification

Having confirmed the first two hypotheses (Section 1.4) and established the fact that a gradient of physical and environmental variables along a river continuum has indeed elicited a graded response from the denitrifying bacterial populations, seasonally as well as spatially, the relative influence of these regulating environmental factors can now be considered in order to substantiate the third hypothesis (Section 1.4).

Field Investigation

Results of the intensive field study of 50 river sites (see Section 6.2) showed that with the exception of the headwater site in the Swale catchment (Ravenseat), where nitrate and organic C availability were low, denitrification was detected at all sites; suggesting that denitrifying bacteria are widely distributed. The occurrence of denitrification everywhere else was surprising in view of the fact that many of the samples had supersaturated dissolved oxygen in the water at the time of sampling and a high proportion of particles in the sediment greater than 600 μm and therefore a very low total carbon content. This once again suggests the occurrence of aerobic denitrification or the existence of anoxic micro-environments in generally aerobic conditions (Lloyd, 1993). The highest denitrification rates were always found in organically polluted lowland rivers, similar to the Wiske, where sediments tended to be covered by decomposing algal or other organic debris.

As with the seasonal studies on the Swale-Ouse and Tweed systems, denitrification rates increased on passing from upstream to downstream in the Tyne, Wear, Tees and Swale. Once again, this trend can be explained by a combination of inter-correlated factors. Moving downstream, current velocity decreases (leading to greater sedimentation) and aqueous nitrate and phosphate increase. In general, sediments become finer (perhaps increasing sites for anoxic environments and microbial attachment) with increased contents of water, C and N.

The rates of denitrification found (<0.005 to 260 nmol N g^{-1} d. wt h^{-1}) are again in the range of values reported for other ecosystems: sandy loam soils, c. 20 nmol N g^{-1} d. wt h^{-1} (Drury *et al.*, 1992); fine-silty soil with values up to

170 nmol N g^{-1} d. wt h^{-1} (Parsons *et al.*, 1991); 24 soils in Northern Ireland with a wide range of physical and chemical properties, 2.5 and 88 nmol N g^{-1} d. wt h^{-1} (Watson *et al.*,1994). Bradley *et al.* (1995) found in an effluent-dominated river values up to 58 nmol N g^{-1} d. wt h^{-1}. In rivers, lakes and estuaries denitrification rates expressed as nitrogen produced per unit weight of sediment are scarce, making comparison difficult.

As previously discussed (Section 1.2), N_2O accumulation depends not only on its synthesis, but also on the extent of its reduction to N_2 by reductase enzymes. 18 % of samples showed negative values for N_2O accumulation, which suggests a net consumption of the N_2O produced, rather than production by denitrification. However, the two other processes that may form N_2O, nitrification and dissimilatory nitrate reduction to ammonia (DNRA) (see Section 1.3), make interpretation of these data difficult. Thus, it is not surprising that in some cases the production of N_2O without acetylene exceeded that with acetylene, should nitrification or DNRA be contributing differentially to the N_2O pool (Firestone & Davidson, 1989; Arah & Smith, 1990). The processes may be expected often to occur simultaneously, though presumably separated spatially and influenced differently by particular environmental factors. Thus, it is not surprising that there was no significant multiple regression between N_2O production and any of the variables.

Estimates of the ratio of N_2O evolved to N_2 evolved gave values up to 115 %. The average was 18 %, with 38 % of samples being over this value. This figure contrasts with other investigations, most previous reports giving values less than 5 % (Seitzinger *et. al.*, 1984; Seitzinger 1988). The generally high proportion found in this study could be due to an incomplete inhibition of nitrous oxide reductase by acetylene and/or inhibition of this enzyme by other factors such as oxygen. The former seems unlikely because the amount of acetylene added was sufficient to achieve complete inhibition (see Section 3.322) and the slurry technique used in this investigation guarantees complete contact between acetylene and the sites of denitrification. In addition, high N_2O production was found in samples with both high and low water content and therefore high and low apparent density. It is probably more likely that oxygen could be an influencing factor, as nitrous oxide reductase is the most oxygen sensitive of all enzymes involved in denitrification (Firestone & Davidson, 1989), and slurries were made with natural river water, which was always over 62 % oxygen saturation. Why samples with otherwise similar characteristics may lead to an increase or decrease in proportions of N_2O remains unclear: no simple or multiple relationship of the N_2O: N_2 ratio with any of the analysed variables was found. A full explanation requires further experimental studies on the influence of individual variables that control the N_2O: N_2 ratio.

In contrast, the variations of denitrification rate in the wide range of sediment and water characteristics presented by the 50 sites was satisfactorily explained (77 %) by multiple regression analysis (see Section 6.22). The application of other models showed similar or lower significance (Table 7.1). Watson *et al.* (1994) found that bulk density and exchangeable Mg^{2+} explained (by stepwise multiple linear regression) 81.4 % of variation for 24 soils collected in Northern Ireland. A boundary-line approach model applied for Saskatchewan chernozemic soils explained 50 % of the variability using field and literature data (Elliot & de Jong, 1993). However, Bergstrom & Beauchamp (1993) were unable to obtain accurate predictions of denitrification rate using the same approach, when considering the air-filled porosity, respiration rate and mineralisable-C content.

Aqueous nitrate and sediment water content explained the greatest amount of variation in the denitrification data from the present study (64 %). A saturation curve relationship between nitrate and denitrification has been reported not only in this study (see Section 6.32), but also in a number of studies on cultures (Koike & Hattori 1975; Oren & Blackburn, 1979), slurries assayed in the laboratory (van Kessel, 1977b) and field data (Andersen, 1977); as nitrate is a substrate for denitrification, this is not surprising. It is less clear to what extent sediment water content was a factor directly influencing the present results. The influence of water content in soils on denitrification rate has been reported in the literature. The addition of water to soil can show a significant increase in denitrification rate (Weier *et al.* 1993): the water content in soils may inhibit oxygen diffusion to soil microorganisms, thus creating an environment favourable for denitrification. As both fineness of soil texture and water content increased, total denitrification also increased. Groffman & Tiedje (1989) suggest that smaller average pore size in finer textured soils may lead to greater soil water retention and greater opportunity to create anaerobiosis. However, it is known that the availability of electrons from organic carbon is in general one of the most important controlling factors in denitrification (Knowles, 1982). In this field investigation, sediment water content was highly correlated ($P<0.001$) with sediment percentage fine particles, C and N contents (see Table 6.3).

The results from this field investigation and the seasonal study suggest that conditions for denitrification probably exist in most streams and rivers with at least some pockets of fine sediments. Further, more controlled, laboratory investigations into the relationships between physical and chemical features of the environment on denitrification were performed in an attempt to clarify the causal and indirect interdependent relationships.

Table 7.1 Proportion of variability in denitrification rate accounted for by various models. AFP = air-filled porosity; DEA = denitrifying enzyme activity; MPN = most-probable-number Counts of denitrifiers; WFP = water-filled porosity; E. Mg^{2+} = exchangeable Mg^{2+}.

Model	Type of sediment	Variables	R^2	Reference
Stochastic	Soils	DEA CO_2 production	$p>0.1$	Parkin & Robinson (1989)
Boundary	Soils	Air porosity Organic carbon Max. air Temp. Nitrate WFP	0.50	Elliot & de Jong (1993)
	Soils	AFP Respiration rate Mineralisable C Nitrate	0.60	Bergstrom & Beauchamp (1993)
Simple regression	Stream sediment	Water soluble C	0.67-0.79	Hill & Sanmugadas (1985)
Multiple regression	Soil	CO_2 Moisture Nitrate Temp MPN DEA	All data set 0.11-0.27 Mean value 0.73-0.91	Parsons *et al.* (1991)
	Soil	E. Mg^{2+} Bulk density	0.81	Watson *et al.* (1994)
	Streams & Rivers	Water content Nitrate	0.64	This study

Laboratory investigations

Nitrate in particular was investigated because of its significant relationship with denitrification found in both the seasonal and 50 site field investigation. Denitrification rate in intact sediment cores responded strongly to the nitrate addition and followed a Michaelis-Menten type kinetic curve (see Section 6.32), as found by other authors for freshwater (Andersen, 1977) and estuarine sediments (Koike *et al.*, 1978; Oren &

Blackburn, 1979; Oremland *et al.*, 1984). The maximum values for denitrification rate in this study involving nitrate enrichment were mostly similar to the maximum rates found in the seasonal study under field conditions. However, comparisons with other studies are complicated by the fact these have been made at a range of temperatures. Ideally, results would be presented for both the field temperature and also standardised to one particular temperature. In the case of the Swale-Ouse, values are comparable because the temperature at the time of the maximum was always within 5 °C of the 15 °C used in the present study.

Apparent maximum velocity showed a marked increase coincident with increasing nitrate concentration on passing down river and it seems almost certain that nitrate concentration is the key factor. Furthermore, these rates were mostly similar to those found in the long-term study on the Swale-Ouse (Section 4.3). However, other factors other than nitrate must be considered when assessing the results, such as number of denitrifying bacteria, their genetic and physiological properties and the optimal conditions of the process. As already discussed, downstream sediment particles were finer, presumably decreasing oxygen penetration and the carbon content of the upper 2 cm of sediment was higher, as already established, all of these variables are likely to favour denitrification.

Apparent half saturation (K_s) concentrations increased on passing downstream and were maximum in the Wiske. This trend agrees with the increasing gradient of nitrate concentration on passing downstream observed in this study and the previous long-term study on the Swale-Ouse (Section 4.22). This would suggest that upstream, where substrate (nitrate) is low, mixed bacterial populations would show the greatest apparent affinity to nitrate (low K_s). At sites with high substrate concentrations, such as on the Wiske, a higher K_s would suggest less efficient reduction of nitrate by denitrifying bacteria. From the results of this study it can be concluded that the mixed populations of denitrifiers at each site are well adapted to the usual levels of nitrate.

It would appear that all previous kinetic studies published refer to experiments with slurries and not intact sediment cores, so caution is needed in comparison of data from this study with previous data. However, the values for maximum velocity at the headwater site and the main river (35.8 to 324 μmol N m^{-2} h^{-1}) (see Fig. 6.8) are well in the range of denitrification rates measured at near ambient conditions for rivers (0 - 345 μmol N m^{-2} h^{-1}, Seitzinger, 1988) and coastal marine sediments (0 - 888 μmol N m^{-2} h^{-1}, Seitzinger, 1988), though denitrification was always detectable in the present study. The uppermost value on the Wiske (860 μmol N m^{-2} h^{-1} in June) fell within this range. K_s concentrations ranged from 13.1 to 90.4 μM nitrate for the headwater site and the main river, but to 460 μM nitrate for the Wiske; these values may be compared with the lower (8 μM) and upper (344 μM) values in previous studies by authors (Koike *et al.*, 1978; Oren & Blackburn, 1979; Oremland *et al.*, 1984; Murray *et al.*, 1989) using the slurry technique.

Another problem in assessing results is the fact that denitrification in the sediment has been related to the nitrate concentration in the overlying water, as previously discussed. Although transport of nitrate into the sediment is likely to be rapid, the lower and variable nitrate concentration in the sediment means that the K_s for the mixed denitrification enzymes has inevitably been overestimated in slurries. However, by using cores the measurements of kinetic parameters obtained from this study should be more realistic, a future step should be to make detailed measurements during the experiment of changes in the nitrate gradient in the sediment.

Net N_2O production occurred at all sites, however, there was not always an increase with increasing nitrate concentration at all sites. In comparison with the literature, the proportions of N_2O related to total N gases ($N_2 + N_2O$) for the highest nitrate concentrations were surprisingly high, reaching 75 % in the Wiske in June (see Table 6.10). The published values for N_2O in aquatic sediments have been obtained from pore water profiles (Sørensen, 1978b) and direct N_2O flux measurements from cores (Nishio *et al.*, 1983; Seitzinger *et al.*, 1984); net N_2O flux is generally less than 2 μmol N_2O-N m^{-2} h^{-1} and less than 5 % of N_2 production (Seitzinger, 1988). Once again, as N_2O production is an intermediate product in at least three processes in the nitrogen cycle, each influenced by a range of environmental factors, it is difficult to explain the high values found here. However, eutrophication is one factor reported to lead to increased N_2O production, as shown in Narragansett Bay (Seitzinger *et al.*, 1983), where N_2O fluxes increased around 10-fold from the relatively unpolluted lower and midbay to the eutrophic upper bay sediments. Another marine study (Seitzinger & Nixon, 1985) showed in a mesocosm that N_2O flux from sediment and the N_2O: N_2 ratio increased markedly in relation to nitrate input. In addition, in a study into the use of wetlands for water quality amelioration, Freeman *et al.*(1997) found a 95 % decrease in N_2O emissions, compared to a control wetland, as a result of decreased nitrate on diverting water inflows for a 20-week period.

The significant positive relationship between nitrate and denitrification in the seasonal and field study and the fact that all sites showed a marked increase in denitrification following the addition of nitrate in the kinetics study shows that any limitation in denitrification due to some other factor, such as concentration of suitable carbon substrates or phosphate, could be relatively unimportant. However, there should be further studies made to assess the effects of such variables.

Results from the best subsets regression of environmental data with denitrification data from all sites from the Swale-Ouse seasonal study (see Section 4.4) showed that nitrate and temperature explained 68 % of the total variance in this data, thus denitrification may be expected to show a seasonal periodicity based on temperature. The seasonal temperature study (see Section 6.31) showed a general linear response of denitrification rate to a rise in temperature (see Figure 6.7), however, the differing results between assays at different times indicate that more data are required before temperature can be included in satisfactory models to predict denitrification rate. Denitrifying bacteria are known to be a heterogeneous group of organisms (Knowles, 1982), so differences in composition may be expected between sites and seasons, potentially resulting in the differences in slopes. There are apparently no values in the literature with which direct comparisons (over the 3 to 30 °C range of this study) may be made, but sediment cores taken in winter from Duffin Creek, Ontario, showed a four-fold increase in nitrate uptake over the range 0 to 20 °C (Hill, 1983). Two accounts in the literature using slurries report the denitrification rate to increase exponentially as a function of the temperature. This was observed over the range 14 to 35.5 °C in Lake Okeechobee, southern USA (Messer & Brezonik, 1983) and 5 to 18 °C for the marine Narragansett Bay (Seitzinger *et al.*, 1984). One study on an estuary (Koch *et al.*, 1992) reported no changes in denitrification rate over the temperature range 0 - 30 °C, but these studies were carried out over a 24 h period, so may possibly have been influenced by substrate limitation at the higher temperatures.

In spite of the strong relationship ($R^2 = 0.91$) between nitrate, temperature and denitrification found in the laboratory study (see Section 6.33), as already explained, the relationship was not so evident for any particular site when using field nitrate and temperature to predict denitrification, this would suggest that other environmental variables could be exerting an influence.

The organic carbon addition study on the Wiske showed that, at least in November 1996, the rate with slurries showed no response (see Section 6.34). This suggests that suitable C substrates were not limiting for denitrification. The total organic C content of the sediment at this site is relatively high C (0.3 - 0.7 %) with the highest values in late summer and autumn. November is the time of year when there is likely to be the greatest input of organic material from breakdown of submerged macrophytes, so similar assays should be made at other seasons. Other results indicate that organic carbon could at times be an important factor influencing denitrification rate. The study of sediment heterogeneity on the Wiske (see Section 4.41) made in April 1996 showed the highest rates of denitrification for an area with overlying plant debris. Cooke & White (1987) found in an 800 m stretch of the River Dorn, England, that denitrification rate showed a three-fold range according to the amount of mineralisable carbon in the sediment; the highest values were associated with fine-grained sediments. Hill & Sanmugadas (1985) reported a strong relationship between nitrate loss from laboratory incubated sediment cores and the water soluble carbon content in the top 5 cm of sediment. As previously discussed, the 50 site survey also showed that denitrification rate was related first to nitrate and second with water content of the sediment; although, the water content showed a high positive correlation with both the C content of the sediment and the content of particles less than 100 µm, suggesting a potential influence from these other factors.

The limited study on oxygen in the freshwater tidal reaches of the Swale-Ouse (see Section 6.35) served to indicate that further studies are necessary in order to produce strong conclusions. The fact that there was a slight increase in denitrification at Naburn Weir and none at Cawood or Selby would suggest the instability of the sediments has a greater affect on the formation of bacterial populations, as previously discussed. A study on oxygen depletion in a more stable environment may produce very different results; for example Andersen (1977) found that denitrification rates in lake sediments were always higher in anaerobic cores.

7.4 Significance of denitrification in the Swale-Ouse

A simple calculation demonstrated by Cooke & White (1987) has been used to estimate nitrate removal by denitrification in order to assess the significance of denitrification in part of the Swale-Ouse system. The estimates were based on data for Catterick Bridge for October 1995 - September 1996. A stretch of 36.2 km (from Catterick Bridge to the confluence with the Wiske, see Fig. 2.2) was considered for the calculation. The assumptions were the average width was 20 m, 70 % of river bed was suitable for denitrification and denitrification and nitrate concentration were uniform. Estimates of denitrification for each month were based on the single *in situ* rates, while nitrate concentration was based on the mean values from the LOIS core data and monthly measurements from this study (minimum of 5 values). The total denitrification rate for the stretch was then estimated for each month. The amount of nitrate removed by denitrification was calculated and expressed as a percentage of the amount that would be exported in the absence of denitrification.

Within the winter months nitrogen loss through denitrification in the mid-Swale was negligible compared with the nitrogen transported down the river because of high flow and low denitrification. However, in late spring and summer, denitrification could remove approximately 5 % (or 0.14 % km^{-1}) of the total amount of nitrogen transported (a peak of 9.0 % removal was calculated for June). This value is in the lower range of values given for various rivers (review by Seitzinger, 1988). Cooke & White (1987) reported a value of 20% for a 35-km stretch of the R. Dorn, UK, and Christensen & Sørensen (1986) 10% for a 1 km stretches of two lowland Danish streams, both studies being made in summer using acetylene inhibition.

Although a lot of assumptions have been made in this calculation, it serves to show that removal of nitrate via denitrification under low summer flows is a component of the total nitrogen budget of the Swale-Ouse that should be considered. Particularly in view of the fact that this calculation is for the upper section of the river; an increase in percentage nitrate removal would be expected further downstream due to higher denitrification rates and increased riverbed area suitable for denitrification.

7.5 Concluding remarks

The present study has shown that there are very clear seasonal and spatial trends in denitrification and nitrous oxide production in the Swale-Ouse and Tweed river systems as a result of the influence of key environmental variables. These trends are a result of the differential influence of these key environmental variables on denitrifying bacterial populations. It can therefore be concluded that all of the original hypotheses (Section 1.4) can be confirmed. Furthermore, it seems that denitrification is potentially a significant component of the total nitrogen budget of the freshwater systems assessed in this study, even in the fast-flowing, meso-oligotrophic reaches of the upper Swale-Ouse system and River Tweed.

In addition, the data gained in the present study will be incorporated into the LOIS data set. This will be utilised to predict and model the implications of inland activities on events in the adjacent estuarine and coastal zone in order to aid in the future policy formulation for the management of the coastal zone (NERC 1994).

SUMMARY

1) This study investigated the trends evident in denitrification and nitrous oxide production in sediments and the key influencing environmental factors within selected river systems of North-East England in the LOIS (Land-Ocean Interaction Study) area of study.

2) Seasonal and spatial trends were evident in both the environmental and denitrification data measured monthly for 1.5 years (August 1995 to December 1996) along the Swale-Ouse system from source in the Yorkshire Dales National Park to tidal limits, south of York.

3) Conductivity, alkalinity, pH and phosphorus and nitrogen fractions all increased with distance from source, mostly exhibiting a peak in the River Wiske, a highly eutrophic tributary of the River Swale.

4) Seasonal fluctuations were observed in flow, temperature, pH and nitrate. Flow and nitrate showed a peak in winter months, whereas temperature and pH (particularly at the upstream sites) peaked in the summer months.

5) Extracted nitrate and ammonium fractions from sediments mirrored the trends of these fractions in the water column. The proportion of sediment carbon and silt generally increased with distance downstream, again with maximum measurements on the Wiske.

6) Denitrification was measured in sediment cores using acetylene inhibition and expressed by unit area of sediment. The N_2O production data were expressed as μmol N_2O-N m^{-2} h^{-1}, i.e. μmol N as N_2O, not N_2O. This format was used in order to permit direct comparison with results for denitrification (N_2O plus N_2), which were expressed in terms of N (not N_2) as μmol N m^{-2} h^{-1}.

7) Denitrification mostly increased with distance from source, rarely exceeding 20 µmol N m^{-2} h^{-1} at the headwater site; although results from a supplementary survey of the freshwater tidal reaches of the Ouse showed a decrease from the tidal limits at Naburn Weir.

8) Denitrification showed a spring (March to May) peak in activity, particularly in the lowland sites, downstream of Richmond, with the highest rates of denitrification measured on the Wiske (883 ± 134 µmol N m^{-2} h^{-1} in May 1996).

9) A high degree of colinearity was evident between environmental variables, although a significant relationship between denitrification, nitrate and temperature was found through multiple regression.

10) A study of spatial heterogeneity of denitrification at one site showed a four-fold difference in rate between different types of sediment substrate.

11) A peak in nitrous oxide production was found in the summer months for the downstream sites with a minimum in winter, similarly to denitrification. The highest production was found on the Wiske (497±184 µmol N_2O-N m^{-2} h^{-1}) in July 1996.

12) The proportion of nitrous oxide release related to the total gases released during denitrification showed great variation; there was a seasonal trend towards higher proportions in summer months compared to winter months.

13) The seasonal and spatial trends evident in both the environmental and denitrification data in the Tweed river system, under a more limited sampling programme, were consistent with those observed in the Swale-Ouse system.

14) The environmental and denitrification data from the Tweed were of a similar order of magnitude to those measured in the meso-oligotrophic, middle reaches of the River Swale.

15) Intensive field and laboratory investigations were carried out in order to clarify the relationships observed in the long-term study between denitrification rate and the chemical and physical characteristics of water and sediments.

16) A survey was made of potential denitrification and N_2O production in sediment slurries at 50 river sites in N-E. England in late summer to investigate the relationship between rates and environmental factors likely to influence these processes.

17) In the 50-site study, denitrification rate ranged from < 0.005 to 260 nmol N g^{-1} d. wt h^{-1}, tending to increase on passing downstream.

18) N_2O production in the 50 site study ranged from negative values (net consumption) to 13 nmol N_2O-N g^{-1} d. wt h^{-1} and accounted for 0 to 115% of the N gases produced.

19) Both denitrification rate and N_2O concentration in the sediments of the 50 sites were correlated positively with nitrate concentration of the water column, water content of the sediments and percentage of fine (< 100 μm) particles in the sediment.

20) An experimental study investigating the kinetic parameters for denitrification, found that sediment cores taken along the Swale-Ouse exhibited a saturation type curve with added nitrate.

21) Apparent affinity (K_s) for nitrate by the mixed populations of denitrifying bacteria increased on passing downstream from 13.1 to 90.4 μM in the main river, but was highest (640 μM) in the Wiske.

22) Estimates of apparent maximum velocity (V_{max}) for denitrification ranged between 35.8 and 324 μmol N m^{-2} h^{-1} in the Swale-Ouse (increasing from upstream to downstream) and was highest in the Wiske (1194 μmol N m^{-2} h^{-1}).

23) N_2O production with added nitrate showed that rates ranged from below the detection limit (0.05 μmol N m^{-2} h^{-1}) at the headwater site (with lowest nitrate concentration) and 27 μmol N m^{-2} h^{-1} at the downstream site (with highest nitrate concentration in the main river).

24) Comparative seasonal temperature curves were made on intact sediment cores from the Wiske and Ivelet Bridge. Denitrification activity per unit are of sediment generally increased linearly with temperature, although the slopes and intercepts were different on the different occasions.

25) A laboratory investigation of the combined effects of temperature and nitrate on denitrification using intact sediment cores from the Wiske and Ivelet Bridge showed a highly significant relationship between denitrification and both variables ($R^2 = 0.91$, $p<0.001$ and $R^2 = 0.79$, $p<0.001$ for the Wiske and Ivelet Bridge respectively).

26) Addition of organic substrates had no detectable effect on denitrification rate in intact sediment cores from the Wiske, although it did lead to a significant decrease in the proportion of nitrous oxide produced as a result of nitrate reduction.

27) Little or no effect on the potential rate of denitrification was observed when sediment slurries from the freshwater tidal reaches of the Ouse were incubated with reduced oxygen.

APPENDIX 1

Land-Ocean Interaction Study (LOIS)

LOIS is a collaborative Community Research Project, the aim of which is to gain an understanding of the passage of materials from land to ocean and ultimately model and predict their impact on the coastal zone of the UK. The information gained from LOIS will aid in future policy formulation for the management of the coastal zone (NERC, 1994).

Due to the multidisciplinary nature of LOIS, it has been divided into four main elements: River-Atmosphere-Coast Study (RACS), North Sea Modelling Study (NORMS), Shelf Edge Study (SES) and Land-Ocean Evolution Perspective Study (LOEPS).

RACS is the largest element of LOIS and forms a combined study into land-sea interactions in the coastal zone and the major fluxes of materials (nutrients, sediments, contaminants) from rivers, estuaries and the atmosphere along the east coast of the UK from Berwick-upon-Tweed to Great Yarmouth. The present study is part of this element.

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