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Attenuation of nitrate in the sub-surface environment

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Steve Killeen

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Executive summary

Nitrate (NO_3^-) is a widespread contaminant in groundwaters and surface waters. In the UK, groundwater nitrate concentrations have been rising in many locations over recent decades, primarily because of diffuse pollution from intensive farming since the mid-twentieth century. Other significant sources include the disposal of organic wastes and urbanisation. Excessive concentrations of nitrate in drinking water have been associated with adverse health effects, while in surface waters excessive concentrations can cause eutrophication. The Nitrates Directive (91/676/EEC) sets a maximum concentration of nitrate in groundwater, irrespective of whether it is used for potable water. It has been supplemented by the Water Framework Directive (2000/60/EC) which requires that all groundwater bodies achieve good status by 2015, with limits on groundwater nitrate concentrations.

Nitrate is commonly thought of as behaving conservatively in the sub-surface environment but under certain circumstances it undergoes a microbially mediated transformation to nitrogen gas (denitrification). This process can be critical for protecting drinking water supplies and surface waters. This report provides a literature review of nitrate attenuation mechanisms as they occur in the sub-surface environment. It discusses the chemical conditions under which attenuation occurs and the hydrogeological environments within which this has been observed.

The processes controlling nitrate attenuation in the soil zone are well understood. However, for the environment beneath this zone, relatively little is known about the prevailing geochemical conditions that determine whether denitrification will take place.

In the UK, evidence of denitrification is mostly limited to confined aquifers, where dissolved oxygen is depleted. The rates of reaction in these confined zones are slow compared to the timescale over which nitrate loads have increased in the last half-century. With the exception of a number of studies on riparian zones, no UK studies appear to cover attenuation of nitrate in shallow groundwater environments. The wide variety of UK aquitard formations has not been studied in depth, but there is some evidence that suggests the geochemistry and hydrogeology of UK tills and other aquitards are potentially conducive to denitrification.

Riparian zones, wetlands and hyporheic zones appear to be zones of effective, if variable, nitrate cycling, primarily because of high fluxes of organic carbon and saturated conditions near to, or within, the soil zone. Very few UK studies cover this topic but most international studies are thought to be broadly applicable to the UK. Hydrogeological conditions which promote shallow groundwater flow are key to determining the extent of attenuation within the riparian zone. If suitable conditions are present, the rate of biodegradation of nitrate in riparian zones is controlled by the availability and reactivity of organic carbon. Seasonal variation in nitrate attenuation depends on plant growth cycles, the depth of the water table, organic carbon inputs and temperature-controlled rates of plant uptake and denitrification activity.

Finally, this report recommends further studies to improve the understanding of how attenuation may affect groundwater nitrate concentrations in the UK. Geochemical surveys of the availability of electron donors may indicate the potential for denitrification

in aquifers. Surveys of the redox conditions of groundwater might indicate in which aquifers denitrification might be occurring and could be based on the recent Baseline series of reports from the British Geological Survey (BGS) and the Environment Agency.

Contents

1 Introduction

1.1 Background

Nitrate (NO_3^-) is a widespread contaminant in groundwaters and surface waters. Elevated concentrations in groundwater are a significant concern in many parts of the world (for example, European Environment Agency (EEA), 2000). In the UK, nitrate concentrations have been rising in many locations over recent decades (Harris *et al.*, 2004; Beeson and Cook, 2004). Indeed, elevated levels were noted as far back as the 1870s (Addiscott, 1996) but the current rising trends have been primarily attributed to diffuse pollution from intensive farming since the mid-twentieth century (Foster and Young, 1980). The Department for Environment, Food and Rural Affairs (Defra, 2002a) estimate that 70 to 80 per cent of nitrates in English surface and groundwaters come from agricultural activities. Direct application of nitrate to land as an agricultural fertiliser, however, is not the only source. Atmospheric deposition, discharge from septic tanks and leaking sewers, the spreading of sewage sludge to land and seepage from landfills can all contribute to the pollutant load (Wakida and Lerner, 2005).

1.1.1 Human health issues

Nitrate is not directly toxic to humans. However, under strongly reducing conditions, such as those in the human gut, it transforms to nitrite. Nitrite ions pass from the gut into the blood stream and bond to haemoglobin molecules, converting them to a form that cannot transport oxygen (methaemoglobin). Excessive consumption of nitrate in drinking water has been associated with the risk of methaemoglobinaemia or ‘blue baby syndrome’ (Fan and Steinberg, 1996), an acute effect that is accentuated under poor sanitary conditions such as sewage contamination or dirty drinking vessels. For this reason, the European Union has set the standard for nitrate in potable water at 11.3 mg N/l (50 mg NO_3/l) (EU Drinking Water Directive, 98/83/EC), unless a derogation has been specifically sought. The World Health Organisation (WHO) recommends the same limit (WHO, 2004). The drinking water limit in the USA, Canada and Australia is 10 mg N/l. The cost of removing nitrates from drinking water supplies to comply with drinking water standards is significant: Dalton and Brand-Hardy (2003) estimated an annual cost to the UK water industry of £16.4 million for the period 1992-1997. In addition to the financial burden of treatment, water resources are lost as boreholes with excessive nitrate concentrations are abandoned (Knapp, 2005).

High concentrations of nitrates (>23 mg N/l) have been shown to induce stomach cancer in animals, including mice and rats. However, epidemiological studies have not identified a causal link between exposure to nitrate and cancer in humans (Mason, 2002; WHO, 2004) nor is there full understanding of the implications of the rodent-human species barrier in extrapolating animal test observations. Further information on health significance can be found in the WHO publication *Nitrates and Nitrites in Drinking Water* (Höring and Chapman, 2004).

1.1.2 Environmental issues

Excess nitrate concentrations can cause eutrophication, which enriches a water body by increasing levels of nutrients such as nitrogen and phosphorus (Mason, 2002). High nutrient levels affect biodiversity by favouring plants which need, prefer or can survive in nutrient-rich environments, and this can lead to excessive plant (typically algal) growth. Low oxygen levels caused by algal respiration or decay may then kill off invertebrates and fish. Certain algal species, such as freshwater cyanobacteria and marine dinoflagellates, produce toxins that can seriously affect the health of mammals, birds and fish (WHO, 1999). Algae can also cause fish asphyxiation by physically clogging or damaging their gills. Eutrophication can also adversely affect a wide variety of water resources used for drinking, livestock watering, irrigation, fisheries, navigation, water sports, angling and nature conservation. It can produce undesirable effects such as increased turbidity, discolouration, unpleasant odours, slimes and foam formation (*www.fwr.org*).

The full impact of eutrophication depends primarily on the balance between nitrogen and phosphorus concentrations in a water body. Where there is excess phosphorus but little nitrogen, small additions of nitrate can lead to changes in the trophic status. In the freshwater environment, excess nitrate particularly affects oligotrophic (nutrient-poor) waters (Mason, 2002) typically found in upland areas in the UK (Palmer and Roy, 2001).

In estuarine and coastal environments (Levine *et al*., 1998), nitrate eutrophication tends to trigger the growth of smothering algal mats across the inter-tidal zone as well as blooms of toxic, nuisance algae (Vitousek *et al.*, 1997). In UK coastal waters, the Mersey Estuary/Liverpool Bay area and Belfast Lough are thought to be showing signs of eutrophication (EEA, 2001). Nitrate imbalance in surface waters can lead to other detrimental effects including acidification. For example, high nitrate levels in runoff from a deforested catchment in the central Amazon Basin led to the leaching of hydrogen ions (H^+) from base cation-poor soils. This in turn mobilised heavy metals to produce toxic conditions in the water courses (Neal *et al*., 1992).

To protect against eutrophication, the EU has set a limit of 11.3 mg N/l for groundwater, irrespective of whether it is to be used for drinking purposes (EU Nitrates Directive, 91/676/EEC). The Nitrates Directive is further supplemented by the Water Framework Directive (2000/60/EC), which requires that all groundwater bodies achieve good status by 2015.

Nitrate is toxic to common eelgrass (*Zostera marina*), even at relatively low concentrations. Eelgrass is an important food and nursery for many commercially important aquatic vertebrate and invertebrate species (*www.ukmarinesac.org.uk, www.marlin.ac.uk*). Nitrite (NO_2^-) is toxic to aquatic animals and the EU guideline concentration for nitrite in rivers supporting salmonid fish is 0.01 mg N/l, while for cyprinids it is 0.03 mg N/l (Freshwater Fish Directive, 78/659/EEC).

1.1.3 Nitrate concentrations in UK groundwater

As a worldwide average, pristine waters contain nitrate at approximately 0.1 mg N/l (Heathwaite *et al.*, 1996). This is extremely low compared to typical modern groundwater concentrations. Studies of UK aquifers suggest that current natural background or baseline concentrations are all less than 5 mg N/l (Table 1.1) – more than an order of magnitude above the global average pristine concentration.

Table 1.1. Baseline nitrate chemistry in selected British aquifers.

Aquifer	Region	Estimated baseline nitrate (mg N/l)	Reference
Bridport sands	Dorset and Somerset	1-3	BGS and EA, 2004b
Chalk	Berkshire	< 4	BGS, 2001
Chalk	Dorset	< 2 (probably < 1)	BGS and EA, 2002d
Chalk	North Downs, Kent and east Surrey	< 5	BGS and EA, 2003a
Chalk	Dorset	< 1.7	Limbrick, 2003
Chalk	Yorkshire and Humberside	< 5	BGS and EA, 2004a
Corallian	Oxfordshire and Wiltshire	< 1	BGS and EA, 2004c
Devonian sandstones	Fife	< 5	BGS, 2001
Granite	Cornwall	< 8	BGS, 2001
Great and Inferior Oolite	Cotswolds	< 3	BGS and EA, 2003b
Lower Greensand	Southern England	1-3	BGS and EA, 2003d
Lower Palaeozoic mudstones	Wales	< 2-3	BGS, 2001
Permo-Triassic Sandstone	West Cheshire and the Wirral	< 3	BGS and EA, 2002b
Permo-Triassic Sandstone	South Staffs. and north Worcs.	< 5	BGS and EA, 2002c
Permo-Triassic Sandstone	Manchester and east Cheshire	< 3	BGS and EA, 2003c
Triassic sandstone	Vale of York	< 4	BGS and EA, 2002a

1.1.4 Prevention of nitrate pollution in England and Wales

The Groundwater Directive (80/68/EEC) lists substances whose introduction to groundwater should be controlled. Two lists are given, where substances on List I must be prevented from entering groundwater, while releases of substances on List II must be limited to avoid groundwater pollution. Nitrate is not a listed substance under the Groundwater Directive, although species that it may form, such as ammonia and nitrite, are included within List II.

Following a number of groundwater public water supplies abandoned because of elevated nitrate levels, a voluntary nitrogen sensitive area (NSA) scheme was established in 1990 to encourage farmers to improve their working practices to reduce nitrate leaching. Delineation of each NSA was based on farmers volunteering fields within catchments of public water supply sources at risk of exceeding, or exceeding, 11.3 mg N/l. Compensation was paid for management practices which minimised future nitrate use and load to receiving waters. The NSA scheme was discontinued and superseded by the (statutory) nitrate vulnerable zones initiative. Groundwater monitoring results from Oxfordshire demonstrate that although the NSA scheme was effective in reducing root zone nitrate leaching, the timescales of groundwater response were only expected to have a noticeable impact after 30 years (Silgram *et al*., 2005).

Under the Nitrates Directive, Defra designated nitrate vulnerable zones (NVZs) (*www.defra.gov.uk/environment/water/quality/nitrate/*). NVZs are areas where the nitrate concentration in surface or groundwaters currently exceeds 11.3 mg N/l or is likely to do so in the future. Groundwater NVZs were defined using a GIS system which integrated aquifer vulnerability, soil type and interpolated groundwater concentrations (Defra, 2002b). Since December 2002, all farmers in NVZs (covering approximately 45 per cent of England and Wales) have been required to implement measures to protect aquifers, such as limiting the application of inorganic nitrogen fertilisers and organic manure. Special measures have also been introduced for the application of organic wastes on sandy or thin soils (*www.defra.gov.uk/environment/water/quality/nitrate/action.htm*). Irrespective of their location with respect to NVZs, all farmers in England and Wales must still comply with statutory codes for protection of water, including the Code of Good Agricultural Practice for the Protection of Water (COGAP) (MAFF, 1998).

1.2 Objectives of this document

In most catchment modelling and assessment approaches, nitrate has been assumed to be a conservative (non-degradable, non-retarded) pollutant. However, nitrate is known to undergo attenuation in the sub-surface, primarily via biodegradation in oxygen-deficient environments (Burt *et al*., 1999; Korom, 1992). There is significant field evidence that high concentrations of nitrate persist in the unsaturated zone in UK aquifers, suggesting that key degradation processes such as denitrification are slow in this part of the environment (BGS, 1999). However, even slow, sustained degradation rates can be effective if source-to-receptor timescales of migration are long. Decade and longer travel times in many aquifer systems provide the necessary rationale to more fully understand sub-surface nitrate attenuation (for example, solute migration times through most UK unsaturated zones of 20-50 m are typically several decades).

This report reviews the literature which describes the processes that affect sub-surface transport of nitrogen as nitrate, although the Environment Agency (2003) deals with nitrogen transport as ammonium. The report draws conclusions on the potential for nitrate attenuation in the sub-surface environment under UK conditions, particularly in aquifers and at the surface water-groundwater interface. The report focuses on processes that operate below the soil zone, out of the reach of plant roots. Soil nutrient cycling or plant uptake rates are not discussed, except where the water table is sufficiently shallow for these processes to directly remove nitrate from groundwater. Within a regulatory context, the Environment Agency will expect site-specific data to be obtained for sensitive hydrogeological environments where hazardous activities are proposed. However, generic information herein may be used for initial risk assessment (risk-screening), where site-specific information is not available.

This report provides Environment Agency officers and other interested parties with information on the conditions conducive to nitrate attenuation. It will be useful for the assessment of pollution risks from agricultural activities, landfills and potentially contaminated land. The literature review provides a summary of the science of nitrate attenuation from which land management practices can be adapted to reduce nitrate pollution in the most financially and technically effective manner. However, more research is needed on the attenuation of nitrate in the sub-surface environment.

Section 2 of the report briefly describes the sources of nitrate in groundwater, while Section 3 outlines the physical transport processes that contribute to the attenuation of nitrate in the sub-surface. Section 4 describes the biochemical reactions and processes that lead to depletion of the nitrate load from groundwater. Section 5 is a survey of observed attenuation and depletion of nitrate in sub-surface environments, while Section 6 summarises information from preceding sections and explores knowledge gaps.

The literature search was undertaken using literature abstracting services and internet data sources. The search procedure is described in Appendix 1. Further information on literature references relevant to nitrate attenuation is provided in Appendix 2.

1.3 Key definitions

Definitions of key biochemical concepts are presented here. A full glossary is provided in the back of this document.

Aerobic	An environment containing molecular oxygen; biodegradation or other process requiring molecular oxygen
Anaerobic	An environment containing no molecular oxygen; biodegradation or other process that does not require molecular oxygen

Oxidising	Conditions favouring oxidative degradation, such as aerobic environments or those where nitrate is a major microbial respiratory substrate.
Reducing	Conditions favouring reductive degradation, such as anaerobic anoxic environments where microbial respiration is generating methane or hydrogen sulphide.

2 Characteristics and sources of nitrate

2.1 Introduction

Nitrate (NO_3^-) is an anion with a molecular weight of 62 g/mol. It does not significantly sorb to aquifer materials under typical sub-surface conditions (Section 3.3). Nitrate is non-volatile and is stable under aerobic groundwater conditions. However, under anaerobic conditions it can be converted to other oxides of nitrogen and to molecular nitrogen by the process of denitrification. Denitrification is the reduction of NO_3^- to NO_2^-, then to NO, N_2O and N_2 (Section 4.2). It is almost always a microbially mediated redox process in groundwater and requires the presence of an electron donor (such as biodegradable organic carbon and/or sulphide minerals).

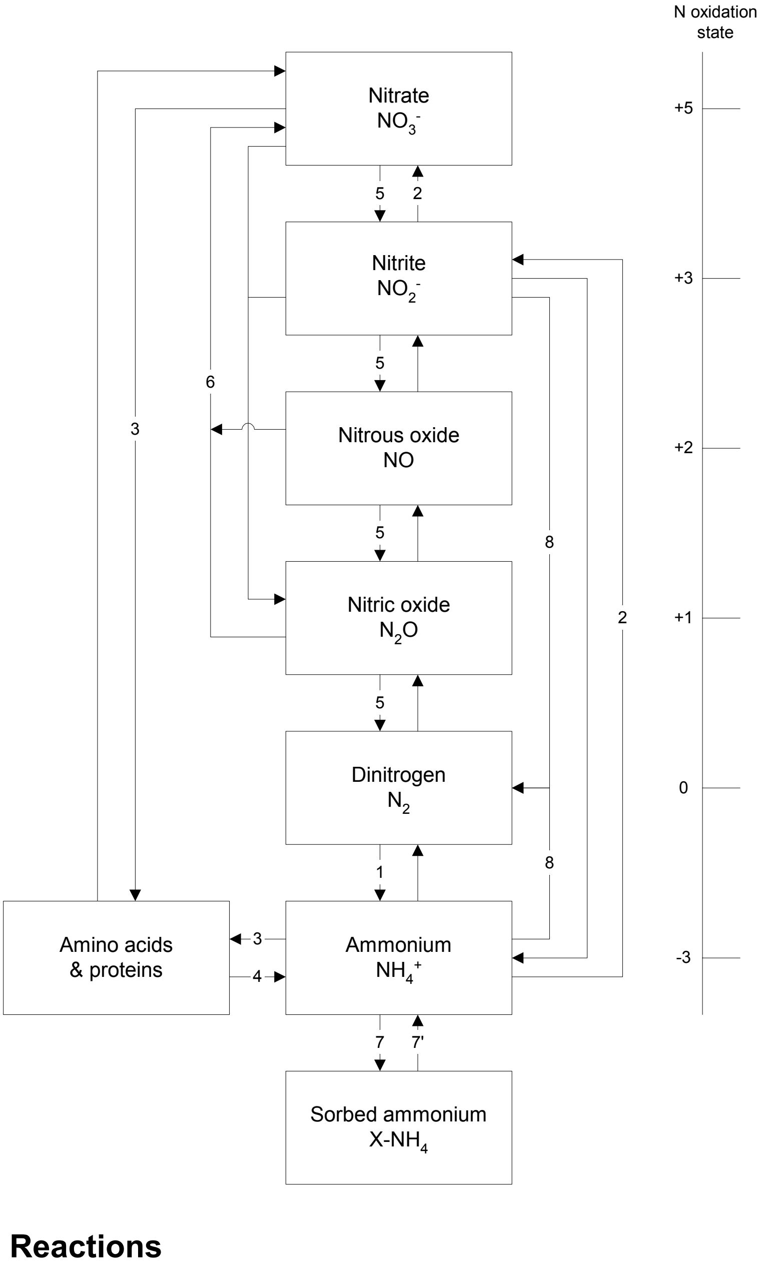
Section 2.2 provides an introduction to the biochemistry of nitrogen transformations in soil and in the sub-surface, following which Section 2.3 discusses the natural and anthropogenic sources of nitrate, with particular emphasis on those related to diffuse pollution. Section 2.4 briefly discusses the geological and pedological controls on nitrate leaching from the soil zone to groundwater.

2.2 The nitrogen cycle

As it moves through the nitrogen cycle (Figure 2.1), an atom of nitrogen may occur in many different organic and inorganic chemical forms, each performing an essential role in the ecosystem. Transformations between these forms mainly involve reactions that reduce or oxidise the nitrogen atom (Section 4.1), and most are microbially mediated. The nitrogen cycle involves the following reactions (Brady and Weil, 2002):

Fixation	Nitrogen is freely available in the atmosphere, but the very stable triple bond of the dinitrogen (N_2) molecule requires considerable energy to break (the activation energy). This can be accomplished by a limited number of bacteria that tend to be symbiotic with plants such as legumes, where the higher plant supplies energy for the reaction from photosynthesis. The nitrogen is converted to ammonium, which may then be assimilated by the plant.
Mineralisation (ammonification)	At least 95 per cent of the nitrogen stored in soils is present within organic compounds that make it insoluble (and therefore not leachable) but leave it unavailable for use by higher plants. Much of the nitrogen is present as amine groups ($R{-}NH_2$), in proteins or as part of humic compounds. Soil micro-organisms convert these to simpler amino-acids, then to ammonium.
Nitrification	Nitrification describes the oxidation of ammonium to nitrite and nitrate. This occurs under aerobic conditions (Environment Agency, 2002).

Denitrification	Denitrification describes the conversion of nitrate and nitrite to nitrogen gas, which can then be lost to the atmosphere from the groundwater/soil system (Section 4.2). Denitrification occurs mostly under anaerobic conditions.
Assimilation (immobilisation)	Assimilation is the opposite of mineralisation and describes the conversion of nitrates and ammonium into organic forms and ultimately biomass.



Reactions

1. Fixation*
2. Nitrification*
3. Assimilation by plants
4. Mineralisation*
5. Denitrification*
6. Nitrate in precipitation (as dilute HNO_3)
7. Adsorption and desorption
8. Anammox*

* Microbially-mediated processes

Figure 2.1. Chemical species in the nitrogen cycle (based on O'Neill, 1985)

2.3 Sources of nitrate in soil and groundwater

2.3.1 Geological nitrate

Organic matter-rich sediments contain relatively high concentrations of organically bound nitrogen, which is mineralised to ammonium as the sediment undergoes diagenesis to form a sedimentary rock (Rodvang and Simpkins, 2001). If this ammonium is nitrified, it can produce high levels of nitrate that are entirely natural. Because of groundwater flushing in aquifers, this nitrate tends only to survive in aquitards. Geological nitrate in North American aquitards is, on average, present at a much higher concentration than that from agricultural pollution: 164 mg N/l and 32 mg N/l respectively (Rodvang and Simpkins, 2001). It can be distinguished from agricultural nitrate because it is usually present at higher concentrations towards the base of the aquitard and is not found in association with elevated levels of tritium. Geological nitrate can also be distinguished by its stable isotope ratio (Section 4.7.1).

No literature appears to explicitly cover the occurrence of geological nitrate in the UK. For example, pore water concentrations of nitrate in the Kimmeridge Clay of Oxfordshire are not particularly high at 1.1 to 3.1 mg N/l (BGS, 2004) despite variably high organic carbon contents (Tyson 2004), suggesting that any original nitrates may have been flushed or undergone in situ denitrification, possibly in association with pyrite oxidation.

2.3.2 Atmospheric deposition

Atmospheric nitrogen originates from a variety of natural and anthropogenic sources, and is deposited on land under both wet and dry deposition. Natural sources include HNO_3 created from nitrogen gas and water vapour by lightning, and natural ammonia emissions from rotting vegetation and manure. Anthropogenic sources include nitrogen oxides (NO_X) from the combustion of fossil fuels (which again may be converted to HNO_3 by lightning), industrial emissions and ammonia volatilisation from manure stores. The natural sources are, however, minor in comparison with the anthropogenic ones, and it can be reliably assumed that almost all rainfall nitrogen in the UK is of anthropogenic origin (Jordan, 1997).

Hayman *et al.* (2001) provide a comprehensive review of recent (1986 – 2000) rainfall deposition of chemical species for the UK. Station averages for the rainfall concentrations of nitrate-N and ammonium-N are given in Table 2.1. Nitrate concentrations, primarily from anthropogenic inputs, range from the lowest in the Highlands of Scotland to the highest in the Midlands, South-East and East Anglia. Concentrations of both nitrate and ammonium have decreased over the period monitored, where mean total N concentration has decreased by around 30 per cent, presumably because of reduced NO_X emissions from power stations. For comparison with the nitrate application rates in Section 2.3.4, the mean total N value over the UK for this period equates to a rate of 8.3 kg N/ha/a (for 1062 mm/a average rainfall for all monitoring stations). The highest mass deposition rate of nitrogen was actually recorded near Windermere, where although the concentration is close to the UK average, there is very high rainfall.

Table 2.1. Station averages for concentrations of N in rainfall 1986-2000 (Hayman *et al.*, 2001)

	Minimum	Mean	Maximum
Nitrate – N (mg N/l)	0.07	0.35	0.78
Ammonium – N (mg N/l)	0.04	0.42	1.74
Total N* (mg N/l)	0.13	0.78	2.44

* Total does not necessarily equal the sum of nitrate + ammonium because some stations only monitored for one determinand.

Goulding *et al*. (1990) found that approximately 10 kg N/ha/a is deposited by precipitation (based on measurements at four experimental farm sites in south east England). However, dry and particulate deposition can increase total nitrogen to 35-40 kg N/ha/a. Since the mean recharge at these sites is 200 mm/a, if all the nitrogen deposited (assumed to be 40 kg N/ha/a) were to move through the unsaturated zone without attenuation or uptake by plants, Goulding *et al*. (1990) calculated that it would reach the water table at a concentration of 20 mg N/l. Although this is unlikely except on thin, bare soil, it may be significant when fertiliser applications are superimposed.

2.3.3 Land use changes

Intensification of UK agriculture between the 1950s and 1970s, partly in response to the Common Agricultural Policy (CAP), saw increasing areas of permanent pasture being converted to tilled land for arable cultivation. Ploughing exposes soil-bound ammonium compounds and organically-bound nitrogen to the atmosphere. These are mineralised to nitrate, which is readily leached by rainfall runoff and infiltration. Table 2.2 presents some nitrate leaching rates for ploughed grassland; for comparison, if 50 kg N/ha were dissolved in 300 mm of recharge, it would yield groundwater at 17 mg N/l.

Table 2.2. Nitrate leaching rates of ploughed grassland (collated in Wakida and Lerner, 2002)

Vegetation type	Nitrate leaching kg N/ha/a	Reference
Temporary grassland on chalk soil	25 – 50	Cameron and Wild, 1984
Temporary pasture	36	Francis *et al.*, 1998
Grass ley	33	McLenaghen *et al.*, 1996
Ploughed grass	93	DoE, 1988
Temporary leguminous pasture	72 – 142	Francis, 1995

In an urban environment, the disturbance of ground by construction can trigger the leaching of soil-bound nitrogen. Wakida and Lerner (2002) estimated the average nitrate load leached from each of three construction sites in Nottingham to be 65 kg N/ha. Soil pore water concentrations beneath the sites reached a maximum value of 116 mg N/l. However, at any one time there is a limited amount of construction occurring across the area of a city, so diffuse urban sources (leaking sewers, contaminated land and NOx from local vehicle emissions) are likely to contribute more N mass to the underlying groundwater. Lerner *et al.* (1999) estimated that the total annual loading of N to groundwater from the Nottingham urban area is 21 kg N/ha.

2.3.4 Fertilisers

Increased leaching of nitrogen from topsoil and the removal of nitrogen by the crop interrupts the natural nitrogen cycle. Leguminous crops (such as clover, vetch, alfalfa, peas or beans) are able to convert elemental nitrogen in the atmosphere to forms of nitrogen useful to other crop types. Soil nitrogen stores can be replenished by periodically growing a leguminous crop and ploughing it into the soil, known as crop rotation. However, this does not necessarily maximise the potential crop yield, and import of nitrogen is still required for continuous cultivation. Nitrogen can be imported as manure, dairy washings or as sewage press cakes; in modern agriculture, however, most nitrogen is imported as mineral fertilisers.

Studies on winter wheat at Rothamsted Research Station, Hertfordshire (Addiscott, 1996) indicate that applied fertiliser nitrogen is not necessarily the direct source of nitrate pollution. Less than 10 per cent of nitrogen applied in the spring is likely to be lost to leaching, because most of the applied nitrogen is taken up by the crop and converted to organic forms. Decaying organic matter in warm and wet autumn soil is rapidly mineralised and nitrified to nitrate and is therefore prone to leaching by winter infiltration. Some of the historic correlation between fertiliser application and nitrate leaching is therefore likely due to the greater amount of organic matter left in the soil after harvest (from increased crop yield). However, if excessive amounts of nitrogen fertiliser are applied to the soil, direct loss of nitrate can result.

Defra (2000) provides detailed guidelines on fertiliser application to agricultural land, along with the relationship between crop yield and nitrogen application, which shows the application rate at which crop yield is maximised (Figure 2.2) and the associated loss of nitrate via leaching is minimised – this tends to be around 200 kg N/ha (Addiscott, 1996). The parameters of the relationship are dependent upon the soil (texture, pH, organic content), the crop variety and its method of planting and fertiliser application (Goulding, 2000). A key result of this relationship is that even at the optimum application rate, significant nitrate leaching losses are predicted (Bhogal *et al*., 1997).

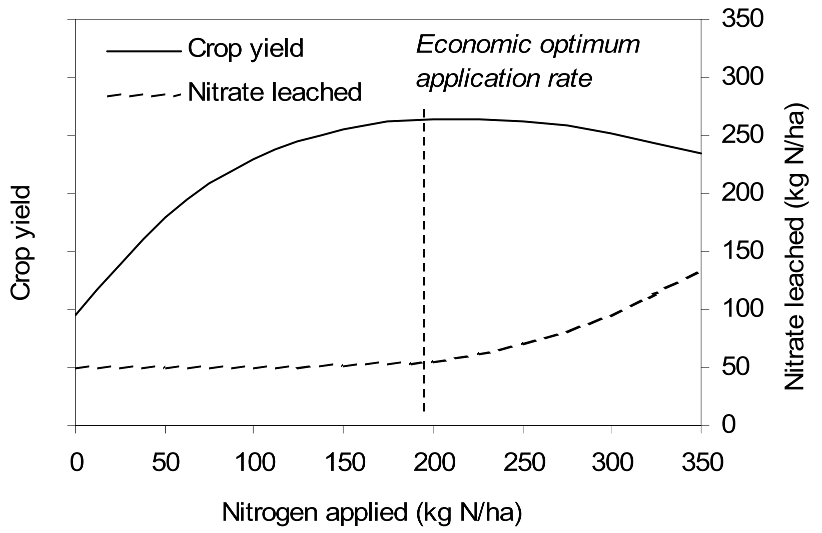


Figure 2.2. A typical crop nitrogen response curve (Defra, 2000) and nitrate leaching losses (based on Addiscott, 1996)

Foster (2000) shows how the use of artificial fertilisers affected British agriculture between 1940 and 1980. A three-fold increase in food production has been accompanied by a 20-fold increase in the use of fertilisers. The remainder of nitrogen has therefore been lost to the atmosphere by denitrification, leached to surface and groundwater as nitrate, or remains stored as a source of nitrate in the unsaturated zone.

Artificial nitrate fertilisers are applied as ammonium nitrate (34% N), ammonium sulphate (21% N), calcium ammonium nitrate (27% N), or urea (46% N) depending on the needs of the crop (Defra, 2000). Most of the ammonium is converted to nitrate in the soil zone. The maximum total (manure plus artificial) application of nitrogen on all crops in Great Britain peaked in the mid-1980s (Defra, 2003). Since that time, there has been an overall decrease in the total application rate (by approximately 17%). In 2002 the average rate of nitrogen application for tillage[1] crops and grassland was 117 kg N/ha (150 kg N/ha for tillage crops and 89 kg N/ha for grass). There were 10.5 million hectares of crops and grassland in Great Britain at this time.

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[1] Tillage is defined as all crops except grass, forestry, glasshouse crops and land designated as 'set-aside' under the Arable Area Payments scheme.

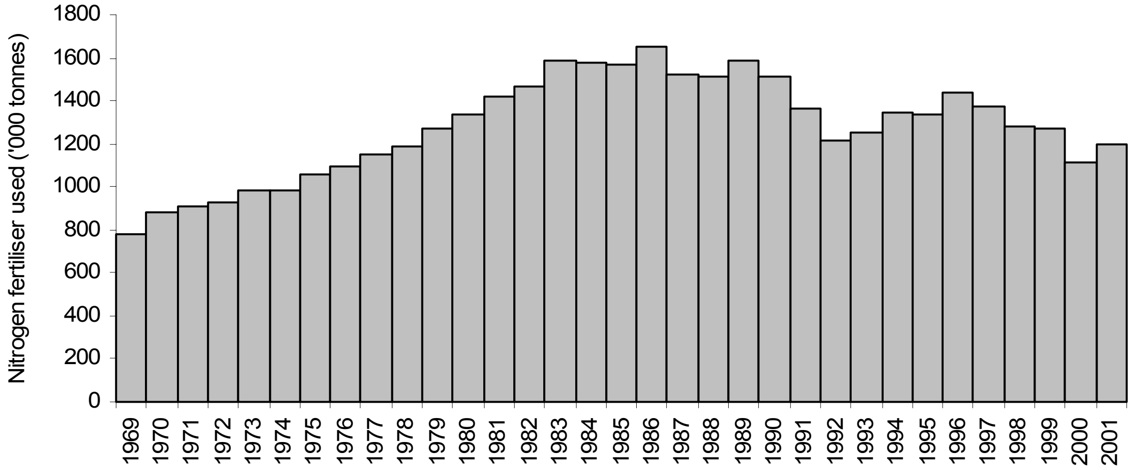

Figure 2.3. Use of inorganic N fertilisers in the UK (*www.environment-agency.gov.uk*)

In 2002, 31% of agricultural land in Great Britain received applications of farmyard manure as fertiliser (Defra, 2003). Solid farmyard manure contains 6 to 7% organically-bound nitrogen, poultry manure between 16 and 30%, and slurries around 1.5 to 5% depending on its water content (Defra, 2000). Depending on the soil type and the timing of the application Defra (2000) estimates that typically 5 to 50% of this nitrogen is mineralised and can be made available for plant growth and leaching.

2.3.5 Point sources

Point sources of nitrogen pollution are commonly associated with a hydraulic surcharge that drives the contaminant into the sub-surface, for example in septic tank soakaways or landfills (Harman *et al.*, 1996; Lyngkilde and Christensen, 1992). These sanitary sources often discharge nitrogen in organically-bound forms [such as urea, $(NH_2)_2CO$, a major component of animal wastes] under reduced conditions; these are usually quickly mineralised to ammonium, and under aerobic conditions this can be oxidised to nitrate.

Industrial sources such as fertiliser production plants may discharge ammonium or nitrate or sometimes both. At points relatively close to industrial sources of nitrogen, nitrate concentrations can be extremely high. For example, Barcelona and Naymik (1984) report on a stock-pile of ammonium and nitrate salts that had been left to weather for three years on a sand and gravel aquifer. In the local groundwater, concentrations of ammonium were up to 1500 mg N/l, with concentrations of nitrate over 300 mg N/l. The plume was approximately 5 ha in the area. Explosives such as TNT are often nitrogen-based compounds and their weathering products yield nitrate. There are a number of nitrate plumes around the world with this origin (Beller *et al.*, 2004).

Urea is often used for de-icing roads and airport runways in locations where rock salt (NaCl) would cause structural corrosion of reinforced concrete or the metal fabric and electronic systems of aircraft. However, this use of urea is being phased out in favour of glycol and calcium chloride and it should not be a nitrogen source in the future. Run-off from the elevated section of the M6 motorway north of Birmingham has been found to contain elevated nitrogen (ammonium) concentrations after application of urea as a de-icer in winter periods. Run-off to the River Tame, which runs adjacent to the motorway, has been found to pollute the receiving waters and detrimentally affect its ecological quality (Ellis, 2003).

Many dispersed point sources can appear to come from one single source of diffuse pollution. Ford and Tellam (1994), for example, suggest that a general increase in nitrogen levels in the Birmingham aquifer may partly arise from ground discharges of nitric acid and nitrate compounds from metalworking industries. Small nitrate plumes from individual septic soakaways can be dispersed in the aquifer to form one observed plume. Similarly, Fukada *et al*. (2004) suggest that sewer leakage beneath UK towns tends to appear as one diffuse source. Lerner *et al.* (1999) estimated that the total annual loading of N to groundwater from the Nottingham urban area is 21 kg N/ha, comprised of leaking mains (37%), leaking sewers (13%), soil leaching (9%) and other sources such as contaminated land and industry (41%).

2.4 Nitrate leaching from soils

Numerous studies have shown that the soil zone can act as both a source of nitrate and a zone of active denitrification (Parkin, 1987; Goulding *et al*., 1993; Bakar *et al*., 1994). Organically-bound nitrogen in the soil is mineralised to ammonium, which is quickly nitrified to nitrate, which is then available for leaching. The soil zone is also the most active area of nitrogen cycling, both by microbial denitrification and plant uptake. Most denitrification within the soil zone probably occurs in the uppermost 10-15 cm, where organic carbon concentrations are greatest from plant degradation and root exudates, and becomes less significant with depth (Burt *et al*., 1999). Deeper rooting plants should, therefore, allow for higher denitrification in addition to allowing for plant uptake from deeper levels. Denitrification rates in agricultural soil are highest in the autumn, when soil is moist but still warm (Addiscott, 1996).

A simple mass balance between the nitrogen applied to a crop and the nitrogen removed at harvest will therefore not provide an accurate estimate of the nitrate leached to groundwater. A nitrate modelling tool, SUNDIAL, has been developed by Rothamsted Research Station to simulate the nitrogen cycle in agricultural soils (Smith *et al*., 1996; *www.rothamsted.bbsrc.ac.uk/aen/sundial/sundial.htm*). The model uses a mass balance of nitrogen (input as atmospheric deposition, seed, mineral fertilisers and manure; output by leaching, denitrification, harvest and volatilisation) to optimise fertiliser requirements on a weekly basis. It may be used by UK hydrogeologists to predict concentrations of nitrate leaching from agricultural regions. Wriedt *et al.* (2005) use a soil leaching model mRISK-N to provide input to a groundwater model which integrates these inputs over a small lowland catchment. mRISK-N also considers the major nitrogen transformations in soil systems and combines these with a soil water balance model.

Soil texture and type affect nitrate leaching rates, with coarse permeable soils allowing more leaching through larger, better connected pore spaces (Goss *et al*., 1998). It is also recognised in the definition of groundwater vulnerability (NRA, 1995) and the designation of NVZs (Defra, 2002b) that sandy soils lead to higher nitrate (and other contaminants) leaching to groundwater than clayey soils. High nitrogen retention in clayey soils (Hubbard *et al*., 2004) can subsequently be released on ploughing. Macropores (such as root holes, worm holes and desiccation cracks) may facilitate bypass flow around the shallow root zone area of most active denitrification. However, Casey *et al.* (2001) and

Jørgensen *et al*. (2004) conversely found elevated denitrification rates where macropores provided a preferential flow path for limiting nutrients, including both nitrate and organic carbon.

Scholefield *et al*. (1996) present linear regressions relating the amount of nitrate applied to a catchment to the peak concentration in river flows (Figure 2.4). Variation in precipitation is accounted for by separating regression coefficients by Environment Agency region. Slopes for river catchments that comprise low-lying limestone or chalk in drier areas, such as Thames, Anglian and Southern regions, were steeper than those for rivers draining upland areas, such as the South West and Welsh regions. This indicates that for a given application rate, a greater river concentration will be expected in a chalk/limestone stream than a river draining a clay area. Regressions were used to predict which nitrate loadings might be expected to give rise to river water concentrations in excess of the Nitrates Directive limit.

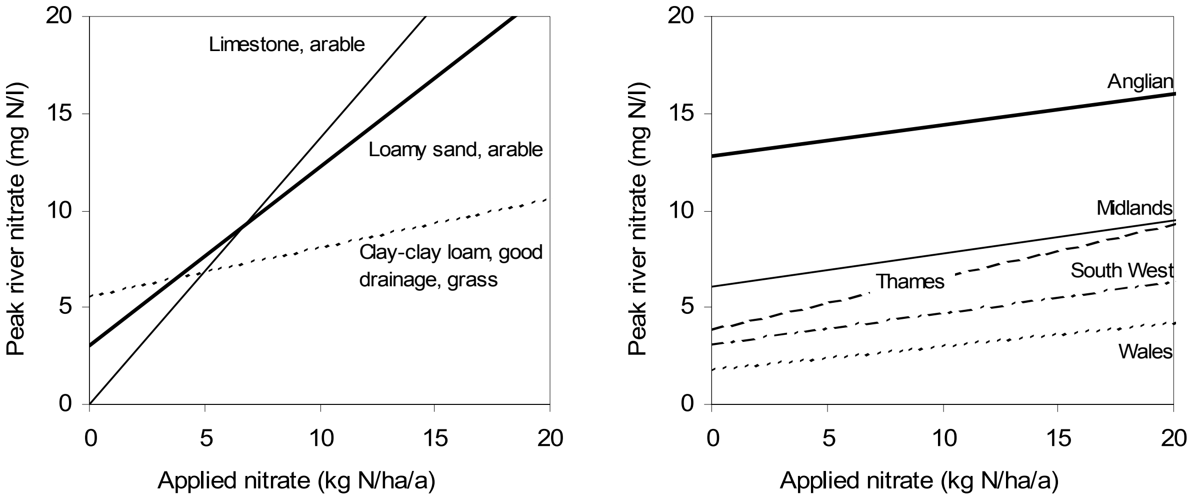

Figure 2.4. Variation in peak river nitrate concentrations for different lithologies and Environment Agency regions (after Scholefield *et al*., 1996)

Carey and Lloyd (1985) used a number of land-use and geology-dependent relationships to model the nitrate leaching source term for a distributed, numerical, nitrate transport model of some 600 km^2 of East Anglian chalk. The land uses were arable (with crop-dependent N uptake), unfertilised grass, fertilised grass and woodland. The simulated source term also accounted for ploughing of grasslands. Potential nitrate leaching rates calculated according to land use were converted to actual leaching rates using an empirical geology-dependent term to account for denitrification in the soil and unsaturated zone. These ranged from one per cent loss by denitrification in thin, clay-free chalky soils to 95 per cent loss in thick clayey soils.

3 Physical transport processes

3.1 Recharge and the unsaturated zone

As a non-sorbing solute, nitrate moves at the same velocity as the water in which it is dissolved. The movement of a solute within the water in which it is entrained is called advection, with the mean advective velocity of solute in flowing groundwater being typically predicted by Darcy's Law, or the Richards Equation in the unsaturated zone (Fetter, 1999). In the unsaturated zone of an aquifer with primary (intergranular) porosity only, pores are not fully saturated and the hydraulic conductivity and effective porosity are scaled down accordingly. With decreasing water saturation, hydraulic conductivity values decrease proportionately more than the effective porosity (Van Genuchten, 1980). Thus, the downward velocity of water (and solutes) in a partly saturated/unsaturated sediment decreases rapidly relative to that if it were fully saturated.

Mechanical dispersion is the process of solute spreading by mixing that occurs at the moving front of a solute plume from lithological heterogeneity at all scales. At the pore scale, this arises from three factors: fluid travels faster through the centre of pores than along the edges (with friction at the grain surfaces);. some fluid parcels travel around grains along longer flow paths; some pores are larger than others and create less friction (Bear, 1972; Fetter, 1999). In fractured rocks, the fluids travel faster through the fractures than through the matrix, causing dispersion over a larger scale. At the outcrop scale, similar processes occur as lithological heterogeneity causes fluids to flow faster through higher permeability zones.

In the unsaturated zone of a fractured porous aquifer, such as some of the UK Permo-Triassic Sandstones, infiltration can move through both the matrix and fractures. In chalk, however, only a fraction of the matrix is sufficiently permeable to allow free drainage, and fractures only conduct water when the matrix permeability is overwhelmed, such as during storms (Price *et al*., 2000; Haria *et al*., 2003). Solutes can therefore be transported either more slowly or more quickly depending on the detailed structure of the rock. As a simplification, however, there is a reasonable body of evidence showing that most of the nitrate moves through UK Chalk and Sherwood Sandstone unsaturated zones in a piston-flow like manner, undergoing only moderate dispersion with the result that the most important zone of mixing (nitrate dilution) occurs beneath the water table in the saturated zone (BGS, 1999).

Slow movement of recharge and solutes through thick unsaturated zones leads to the accumulation of nitrogen in the unsaturated zone. Foster and Bath (1983), for example, describe a site where more than 1100 kg N/ha was stored in the top 8 m of the unsaturated zone, and at concentrations in excess of 75 mg N/l, which was moving slowly downwards. BGS (1991) measured an increase in this store to almost 1300 kg N/ha, and identified another site where the store had previously exceeded 2000 kg N/ha. Unsaturated zone pore water nitrate concentrations are typically in the range 20-100 mg N/l (BGS, 1999).

3.2 Transport in groundwater

Advective transport dominates the transport of solutes in aquifers, and like all solutes undergoing advection, nitrate is subject to hydrodynamic dispersion and diffusion. Dispersion is the process of solute spreading by mixing that occurs at the moving front of a solute plume, driven by pore-scale and macro-scale heterogeneity in the porous medium. Diffusion occurs because of a gradient in solute concentration; it is the dominant process in low permeability porous media driven by low advective velocities (Rowe *et al.* 1988), but is relatively insignificant as a transport process in non-fractured porous aquifers. Perry and Green (1998) present the free water diffusion coefficient of dilute nitric acid as 2.98×10^{-9} m^2/s.

In fractured, porous aquifers such as chalk, solute movement is primarily by advective flow through fractures, but is attenuated by diffusion into the matrix (Foster, 1993). This has two effects on solute breakthrough (Figure 3.1).

Firstly, the initial arrival of the solute pulse is delayed as solute diffuses from the high-concentration solution in the fracture into the low-concentration pore water. Solute migration in the fracture is therefore retarded relative to the advective transport of water. The concentration in the fracture itself is depleted by this diffusion and can be very low at the fracture outlet (Figure 3.1A).

Secondly, after the solute pulse has passed, while the fracture is being flushed with fresh water the solute diffuses back out of the primary porosity into the fracture. This acts as a secondary source that returns solute into the flow system over a much longer period (Figure 3.1B).

These effects are also observed in porous media with connected macropore features (such as root and worm holes, desiccation cracks). They are commonly observed in near surface soils and subsoils where such features remain open – in other words, not closed from overburden (Brady and Weil, 2002). McKay *et al*. (1993) measured solute transport in a clay till through fractures down to approximately 6 m, beneath which the fractures were closed from overburden pressure. In contrast, Gerber *et al*. (2001) identified fractures and sub-vertical sandy ‘dykes’ throughout the depth of a 60 m thick till aquitard. Gerke and Van Genucthen (1993) observed dual porosity effects in even seemingly homogeneous coarse-grained materials.

Jørgensen *et al.* (2004) show that at low flow rates in a macroporous (fractured) till, nitrate is retarded relative to a bromide tracer. This may be caused by the dual porosity effect and the relative diffusion coefficients of nitrate and bromide (the diffusion coefficient of nitrate being approximately twice that of bromide). However, even bromide is retarded by matrix diffusion relative to a non-diffusing colloid phase (McCarthy *et al*., 2002).

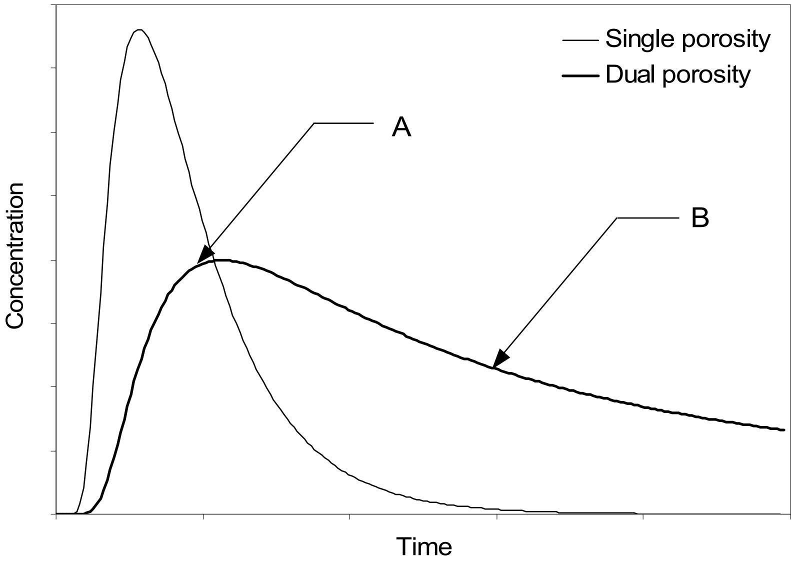

Figure 3.1. Example breakthrough curves for single and dual porosity media. Single (intergranular) porosity solution from Domenico and Schwartz (1997), dual porosity solution from Tang *et al.* (1981)

3.3 Sorption

Cations (positively-charged ions) are readily sorbed or exchanged to negatively-charged mineral surfaces (such as clays). Sorption of anions (negatively-charged ions) under typical groundwater conditions is more complex and tends to only occur to specific species (such as phosphate and to a lesser extent, sulphate) (Stumm, 1992) and is therefore less commonly observed in groundwater. Sorption of the halides (such as Cl^-, Br^-) and nitrate is not usually observed. However, sorption of nitrate and chloride has been noted in soils that contain allophone, imogolite and other poorly-crystallised oxide or hydroxide materials (Katou *et al.*, 1996). These minerals, however, tend only to be found in soils. Clay *et al.* (2004), for example, showed that nitrate was retarded relative to bromide in a smectitic clay-loam soil (the retardation factor for nitrate was approximately 1.37). We are not aware of any groundwater studies that have observed nitrate sorption[2].

Sorption of nitrite in soil is, however, commonly observed (Davidson *et al.* 2003; Fitzhugh *et al.*, 2003). As an attenuation mechanism for nitrate, this requires that nitrate is firstly converted to nitrite via denitrification (Section 4.2). Davidson *et al.* (2003) hypothesise that the nitrite reacts with the aromatic ring structures of dissolved organic matter to produce dissolved organic nitrogen compounds. These may then be adsorbed to soil or taken up by plants and bacteria.

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[2] It should be noted that soil scientists tend to use the term ‘retention’ rather than ‘sorption’ or ‘retardation’.

4 Processes leading to nitrate depletion

The principal mechanism for the depletion of nitrate concentrations in groundwater is microbial denitrification; this chapter focuses on a description of that process in the sub-surface. Other microbial processes can also deplete the nitrate load in groundwater and are briefly considered (Section 4.9).

4.1 Introduction to redox chemistry relevant to nitrate attenuation

4.1.1 Background

Bacteria in aquifers obtain energy from the oxidation of organic or inorganic compounds (such as FeS_2, Fe^{2+}, Mn^{2+}). Bacteria that use organic carbon as the energy source also tend to use it as a source of cellular carbon (heterotrophism), while those that use inorganic compounds will normally use inorganic carbon (mainly from HCO_3^-) for cell construction (autotrophism).

Bacteria obtain their energy by mediating chemical reactions which often involve the transfer of electrons between compounds (Section 4.1.2). They therefore need an electron donor and to balance the oxidation-reduction (redox) reaction, an electron acceptor. Figure 4.1 shows the fate of organic matter (probably the most common electron donor in aquifers) in the presence of a variety of electron acceptors that commonly occur in the sub-surface. Organic carbon tends to be oxidised preferentially by the electron acceptor that supplies most energy to the micro-organisms, namely oxygen. With an excess of organic carbon, aerobic bacteria use dissolved oxygen until it is depleted. Once oxygen concentrations are depleted, reduction of other electron acceptors becomes energetically favourable.

Once oxygen is consumed, facultative anaerobes - bacteria that are capable of surviving with or without oxygen - use nitrate as an electron acceptor. As oxygen levels decrease, obligate anaerobes - bacteria that survive only in the absence of oxygen - begin to use the remainder of the available electron acceptors and when the nitrate is depleted, reduction reactions proceed through manganese and iron oxides, then sulphate. This sequence of redox reactions is commonly seen along flow lines in aquifers (Edmunds *et al*., 1982; Bishop and Lloyd, 1990) and in landfill leachate plumes (Christensen *et al*., 2000; Bjerg *et al*., 1995; Lyngkilde and Christensen, 1992). The boundary where redox conditions rapidly change (usually from oxidising to reducing conditions) is called the 'redoxcline' (Postma *et al*., 1991). However, natural processes seldom have such strict boundaries and a number of redox reactions may occur simultaneously in any one block of aquifer (Ludwigsen *et al*., 1997; McGuire *et al*., 2002). This is often because redox reaction rates tend to be slow, and it is unlikely that a complex system such as a landfill leachate plume is at equilibrium with respect to redox (Christensen *et al*., 2000). Microbial communities in biofilms can also use pore-scale heterogeneities to undertake a range of different redox processes in close proximity. Once established, biofilms can locally control redox conditions and allow a range of redox processes that would otherwise not occur at that location. Hence the common use of more vague terms such as ‘oxidising’ or ‘reducing’ that describe the general redox chemistry of a groundwater.

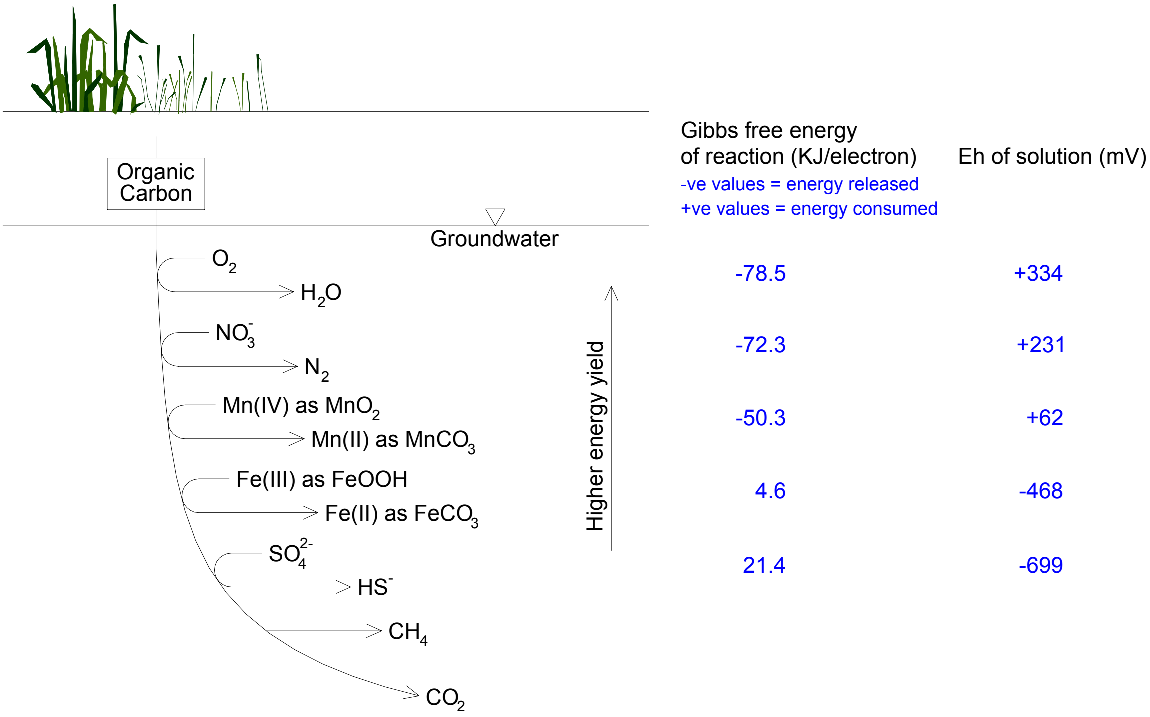


Figure 4.1. Thermodynamic sequence of electron acceptors for oxidation of organic carbon in the saturated zone (adapted from Korom, 1992).

In a typical groundwater, the concentration of sulphate is usually many times greater than that of nitrate because of the dominance of natural, and sometimes anthropogenic, sources. In certain lithologies, oxidised iron and manganese minerals are also abundant and these can participate in the redox reactions. Thus, reduction of sulphate (or Fe and Mn oxides depending on abundance) is often the more important anaerobic redox process in a groundwater system. Denitrification is therefore sometimes dealt with only briefly in the study of regional hydrochemical distribution, as the zone of denitrification can be narrow and may not be detected in boreholes that are widely spaced (and have insufficient resolution) around the zone of denitrification.

4.1.2 Quantifying redox chemistry

Although redox chemistry can be described in purely qualitative terms, a full understanding of the processes requires background knowledge of the energy transfers involved and the quantities used to describe these. This section aims to describe these, but more detail can be found in Appelo and Postma (1993) or Langmuir (1997). As an example of a redox process, consider the following reaction between organic carbon and nitrate:

$$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O$$

In this reaction twenty electrons are transferred from the carbon compound to reduce NO_3^- to N_2. As described above, the carbon compound is the electron donor and nitrate the electron acceptor. Redox reactions are often conveniently split into two half reactions, in this example (e^- representing an electron):

$$4e^- + 5H^+ + HCO_3^- \rightarrow CH_2O + 2H_2O \qquad \text{and,} \quad NO_3^- + 6H^+ + 5e^- \rightarrow \tfrac{1}{2}N_2 + 3H_2O$$

The gain in energy obtained when transferring electrons between the donor and acceptor is given by the Gibbs free energy (ΔG) (Appelo and Postma, 1993). The Gibbs free energy can be converted to an electrical potential difference or voltage (E, volts). So the theoretical voltage corresponding to each half reaction (Eh, volts) is given by the following (Langmuir, 1997):

$$Eh = E^0 + \frac{RT}{nF}\ln\left(\frac{\left[HCO_3^-\right]\cdot\left[H^+\right]^5}{\left[CH_2O\right]}\right) \qquad = 0.036 + 0.0148\log\left(\frac{\left[HCO_3^-\right]\cdot\left[H^+\right]^5}{\left[CH_2O\right]}\right)$$

$$Eh = E^0 + \frac{RT}{nF}\ln\left(\frac{\left[NO_3^-\right]\cdot\left[H^+\right]^6}{\sqrt{\left(P_{N_2}\right)}}\right) \qquad = 1.24 + 0.0118\log\left(\frac{\left[NO_3^-\right]\cdot\left[H^+\right]^6}{\sqrt{\left(P_{N_2}\right)}}\right)$$

Where

E^0 = the standard potential for the half reaction (at standard temperature and pressure, with all reactants present at unit activity)
R = the gas constant (8.314 $J.K^{-1}.mol^{-1}$)
T = the temperature (K)
n = the number of electrons being exchanged
F = Faraday's constant (96 485 C)
[ ] = activity of the species ($mol.l^{-1}$)
P = partial pressure of a dissolved gas (bar)

Other important half reactions involve the reduction of iron oxides and of reduced sulphur. Table 4.1 lists these half reactions and computed equilibrium values. Reduction processes have to be coupled to an oxidation process to yield energy, and the redox reactions with the greatest energy yield are those with the standard potentials of their half reactions farthest apart. Hence, reactions A + K (the oxidation of organic matter by molecular oxygen) will yield the greatest energy. Energy yields are tabulated in Figure 4.1 for the oxidation of organic carbon by various reactants.

Table 4.1 Standard potential, E^0 and Eh at pH 7 and 25ºC of relevant redox half reactions, assuming thermodynamic equilibrium for conditions listed on the table (Langmuir, 1997).

	Half reaction **Reduction →** **← Oxidation**	**E^0 (mV)**	**Eh (mV)**	**Assumptions**
A	$4H^+ + O_{2(gas)} + 4e^- = 2H_2O$	+1230	+816	$P_{O2} = 0.2$ bar
B	$NO_3^- + 6H^+ + 5e^- = ½N_{2(gas)} + 3H_2O$	+1240	+713	$[NO_3^-] = 10^{-3}$ mol/l $P_{N2} = 0.8$ bar
C	MnO_2 (pyrolusite) + $4H^+ + 2e^- = Mn^{2+} + 2H_2O$	+1230	+544	$[Mn^{2+}] = 10^{-4.74}$ mol/l
D	$NO_3^- + 2H^+ + 2e^- = NO_2^- + H_2O$	+845	+431	$[NO_3^-] = [NO_2^-]$
E	$NO_2^- + 8H^+ + 6e^- = NH_4^+ + 2H_2O$	+892	+340	$[NO_2^-] = [NH_4^+]$
F	$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$	+975	+14	$[Fe^{2+}] = 10^{-4.75}$ mol/l
G	$Fe^{2+} + 2SO_4^{2-} + 16H^+ + 14e^- = FeS_2$ (pyrite) + $8H_2O$	+362	-156	$[Fe^{2+}] = 10^{-4.75}$ mol/l $[SO_4^{2-}] = 10^{-3}$ mol/l
H	S^0 (rhombic) + $2H^+ + 2e^- = H_2S_{(aqueous)}$	+144	-181	$[H_2S] = 10^{-3}$ mol/l
I	$SO_4^{2-} + 10H^+ + 8e^- = H_2S_{(aqueous)} + 4H_2O$	+301	-217	$[SO_4^{2-}] = [H_2S]$
J	$H^+ + e^- = ½H_{2(gas)}$	0	-414	$P_{H2} = 1.0$ bar
K	$HCO_3^- + 5H^+ + 4e^- = CH_2O + 2H_2O$	+36	-482	$[HCO_3^-] = [CH_2O]$

Theoretically, the Eh determines the distribution of all redox equilibria in a solution in a similar way to pH expressing the distribution of acid-base equilibria (Appelo and Postma, 1993). Unlike pH, however, Eh cannot be measured unambiguously in most natural waters. Although waters from oxidised environments generally yield higher Eh values than those from reducing environments, it is difficult to measure Eh in any meaningful way to be used in the Nernst equation. There are two reasons for the large discrepancies: lack of equilibrium between redox couples in the same water sample, and analytical difficulties in measuring with a platinum electrode. Although the absolute value of Eh of a solution is not analytically useful, it may be found to be of use in a regional study where relative values from different sample points may correlate to show changes in the redox chemistry. A good example is shown in Figure 5.2 for the Lincolnshire Limestone.

pe is the negative logarithm of the electron activity, like pH is the negative logarithm of the proton (H^+) activity. The electron activity should not be interpreted as a concentration of free electrons in solution, since electrons are only exchanged, but rather as the tendency of a half reaction to release or accept electrons. It is defined as:

$$pe = -\log [e^-]$$

Just as for Eh, high positive values of pe indicate oxidising conditions and low negative values indicate reducing conditions. There is a simple relationship between Eh and pe (Appelo and Postma, 1993):

$$Eh = 0.059\ pe$$

Both Eh and pe are commonly used in literature, pe being used because the logarithmic form of the Nernst equation is easier to deal with algebraically. However, pe is not directly measurable in solution, whereas Eh is.

4.2 Denitrification

Denitrification is the process whereby nitrate is converted, via a series of microbial reduction reactions, to nitrogen gas (Figure 4.2). It can also be reduced to nitrite and nitrous oxide gas by abiotic reactions (Section 4.5.8), but in the sub-surface these reactions are minor in comparison with biological denitrification. The organisms that contribute tend to be ubiquitous in surface water, soil and groundwater (Beauchamp *et al*. 1989); they are found at great depths in aquifers (for example, Francis *et al*., 1989: nearly 300 m below ground). Denitrifiers are mostly facultative anaerobic heterotrophs, so they obtain both their energy and carbon from the oxidation of organic compounds. However, some denitrifying bacteria are autotrophs, so obtain their energy from the oxidation of inorganic species. In general, the absence of oxygen and the presence of organic carbon, reduced sulphur or iron facilitate denitrification.

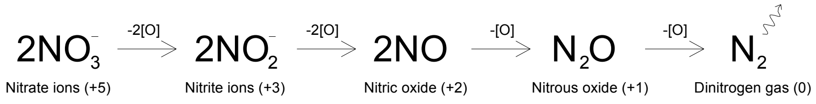


Figure 4.2. Denitrification reaction chain. Numbers in brackets refer to the valence state of the nitrogen at each step (after Brady and Weil, 2002).

The nitrate reduction reaction can be written as a half-equation that illustrates the role of electron (e^-) transfer in the process and is non-specific to the electron donor (Tesoriero *et al*., 2000):

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O$$

Stoichiometric equations that include the electron donors are presented in Section 4.4.

4.3 Transformation products

Although the denitrification process has a stable endpoint at nitrogen gas, the process can be arrested at any of the intermediate stages (Figure 4.3), by a number of factors. This is of key importance, since nitrite is significantly more toxic than nitrate (WHO, 2004). Furthermore, although nitrogen gas is benign, the nitrogen oxides are environmentally harmful. The other product of the denitrification reaction is the oxygen rejected at each step, typically as the bicarbonate ion, carbon dioxide or the sulphate ion. This section discusses these reaction products.

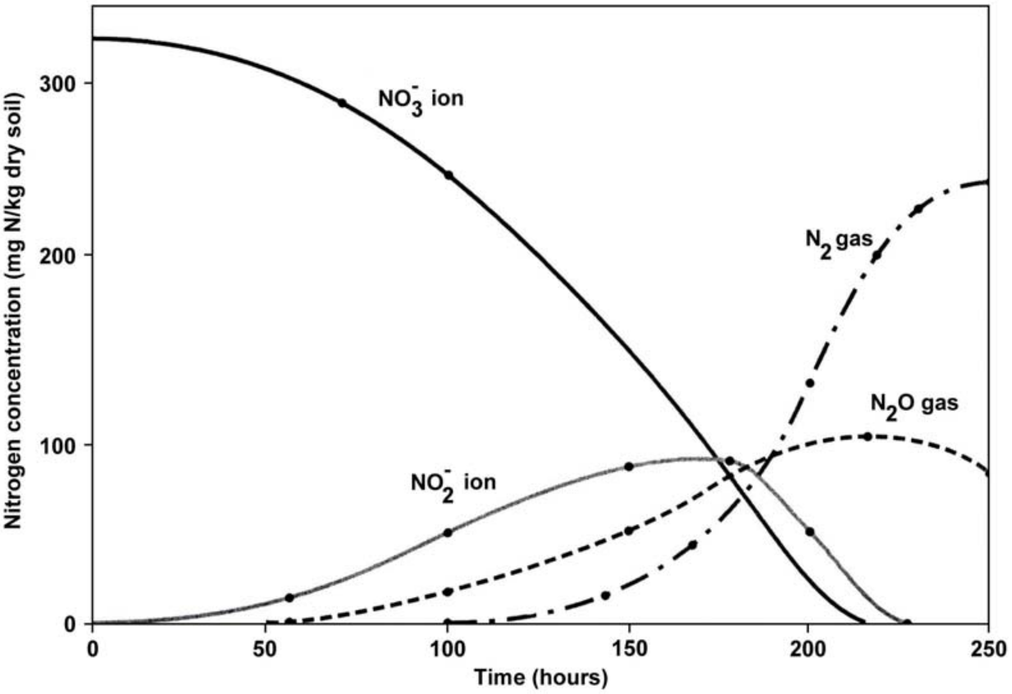


Figure 4.3. Changes in forms of nitrogen during the process of denitrification in a moist soil incubated in the absence of atmospheric oxygen (Brady and Weil, 2002).

4.3.1 Nitrite (NO_2^-)

Nitrite is significantly more reactive than nitrate in the sub-surface. There are a limited range of redox conditions under which it is stable. In particular, the action of the nitrite reductase enzyme is more sensitive to oxygen concentrations than that of nitrate reductase. Nitrate is used preferentially to nitrite by denitrifiers even when both enzymes are present, and a build-up of nitrite may occur due to the time-lag between the onset of reduction of nitrate and the subsequent onset of nitrite reduction (Gale *et al.*, 1994).

In natural waters nitrite rarely occurs at concentrations comparable to those of nitrate, except temporarily under reducing conditions. It also readily reacts with dissolved organics to form dissolved organic nitrogen compounds (Davidson *et al.*, 2003), especially in low pH environments where nitrous acid (HNO_2) is the key reactant. In the Environment Agency Groundwater Monitoring Network and the BGS/EA Baseline groundwater studies (BGS and EA, various), nitrite typically occurs at concentrations of two to five orders of magnitude lower than those of nitrate.

4.3.2 Nitrogen oxides (NO and N_2O)

Nitric oxide (NO) and nitrous oxide (N_2O) are environmentally harmful gases that are formed as part of the denitrification processes, but in favourable conditions, turn rapidly to the environmentally benign form of nitrogen gas. Both of these gases contribute to acid rain, promote the formation of ground level ozone and contribute to global warming; N_2O also destroys ozone in the upper atmosphere. N_2O is equally produced as an intermediate product in the nitrification of ammonium (Environment Agency, 2003); this process is the main contributor to N_2O emissions from UK chalk groundwater (Hiscock *et al.*, 2003), rather than denitrification.

Free nitric oxide is rarely observed because its transformation to nitrous oxide is very favourable under typical environmental conditions. It is usually only observed in small-scale laboratory studies as an intracellular intermediate (Scheible, 1993). Under conditions of very high nitrogen loading (1500 mg N/l ammonium, 300 mg N/l nitrate) in the plume studied by Barcelona and Naymik (1984), however, nitric oxide was suspected to be present.

When oxygen levels are very low, nitrogen gas is the end product of the denitrification process, but where oxygen levels are more intermediate, patchy or variable, the reactions may be arrested at the formation of nitrogen oxide gases (Brady and Weil, 2002). Very high nitrate concentrations or low pH values also arrest denitrification at the N_2O stage. N_2O is often used in wetland studies as an indicator that denitrification is taking place (Delaune and Jugsujinda, 2003; Bernot *et al.*, 2003). Formation of N_2 can be arrested in experimental studies by applying an excess of acetylene ($HC \equiv CH$), so that all denitrified nitrogen can be measured as N_2O. However, the presence of N_2O as an indicator of denitrification is not necessarily conclusive: it can also be derived from partial nitrification of ammonium (BGS, 1999).

Although it may be arrested at the N_2O stage, the denitrification process can be reactivated further along a surface or groundwater flow line. For example, LaMontagne *et al.* (2002) studied an estuarine environment in which groundwater supersaturated with N_2O enters, but is converted to nitrogen in anoxic benthic sediments.

4.3.3 Nitrogen gas (N_2)

Few studies look specifically at the concentrations of nitrogen gas in a system, because effects can be obscured by atmospheric nitrogen, especially in wetland or hyporheic zone systems. However, some studies use the parameter ‘excess nitrogen’ (that is, the N_2 concentration above that expected from equilibration with the atmosphere) to identify denitrification (Vogel *et al.*, 1981). Vogel *et al.* (1981) and Fontes *et al.* (1991) both use this technique to quantify denitrification in groundwater in deep, confined, African aquifers where the groundwater is shown to be several thousand years old. Denitrification was shown to account for up to 22 mg N/l and 46 mg N/l respectively, both very high natural concentrations.

4.3.4 Oxygen-bearing by-products (HCO_3^-, CO_2 and SO_4^{2-})

The fate of the oxygen rejected at each step of the denitrification process depends on the electron donor in the reaction. If organic carbon is the electron donor, the oxygen forms the bicarbonate ion (HCO_3^-) and carbon dioxide (CO_2); if a sulphide mineral is the electron donor, the sulphate ion (SO_4^{2-}) is formed.

Some positive feedback is observed in the denitrification process as the production of bicarbonate and carbon dioxide help to buffer the groundwater pH around neutral conditions, which are most favourable for the denitrification process (Section 4.5.4). Neutral and basic conditions also favour the release of N_2, rather than N_2O.

4.4 Electron donors

4.4.1 Organic carbon (heterotrophic denitrification)

The electrons needed for denitrification can originate from the microbial oxidation of organic carbon. A lack of organic carbon to provide energy to denitrifiers is usually identified as the major factor limiting denitrification rates (Starr and Gillham, 1993; Pabich *et al.*, 2001; DeSimone and Howes, 1998; Devito *et al.*, 2000; Jacinthe *et al.*, 1998; Smith and Duff, 1988). Various stoichiometric equations (depending on the expression of the organic matter in the equation) may be written for the denitrification process relating nitrate and organic matter (carbon) reaction, (for example, Korom, 1992; Jørgensen *et al.*, 2004), representation by the latter being:

$$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O$$

This stoichiometry implies that 1 mg C/l of dissolved organic carbon (DOC) is capable of converting 0.93 mg N/l of nitrate all the way to nitrogen gas. When comparing concentrations of DOC with nitrate, it should be remembered that DOC is oxidised first by dissolved oxygen, the stoichiometry of which is that 1 mg C/l DOC converts 2.7 mg O_2/l. An air-saturated groundwater (10.3 mg O_2/l at 12ºC) therefore uses up approximately 3.8 mg C/l before denitrification can commence. These calculations assume complete coupling, and do not account for bacterial death or C and N release back into the system.

Many factors are known to affect the complex reactivity of soil, or organic matter, towards oxidants, including environmental conditions (pH, temperature and oxidant concentrations), physical protection (sorption to mineral surfaces), and chemical composition (Hartog *et al.*, 2004 and references cited therein). The rate of denitrification is most often related to the amount of DOC in porewater or groundwater, or the amount of soluble organic carbon rather than the total amount of solid organic carbon present (though the two may correlate). Burford and Bremner (1975), for example, correlate the denitrification capacity of soils with the amount of water soluble carbon and mineralisable (bioaccessible) carbon (Figure 4.4). Similarly Cannavo *et al*. (2004) also relate denitrification activity to the concentration of DOC (see Section 5.1.2).

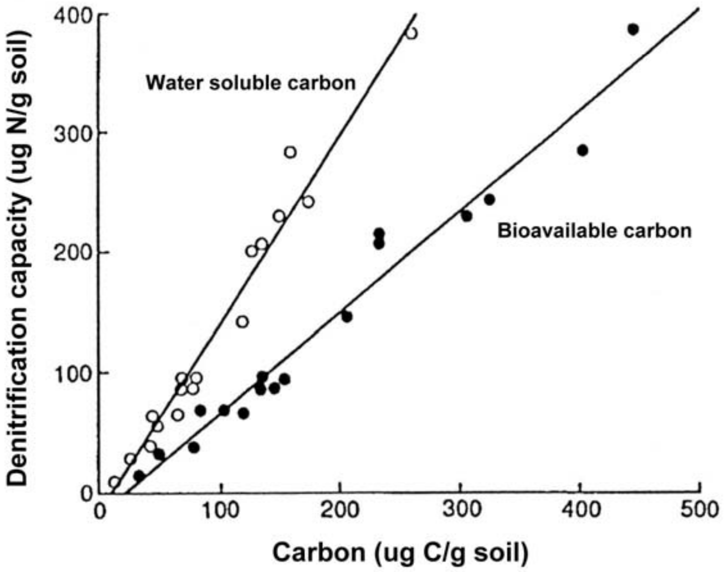


Figure 4.4. Relationship between denitrification capacity of soil and content of water soluble and bioaccessible carbon (from Burford and Bremner, 1975).

Table 4.2 presents indicative values for dissolved organic carbon in UK aquifers, compiled from the Environment Agency database for samples to mid-2003.

Table 4.2. Indicative DOC (Environment Agency database) and f_{OC} values (Steventon-Barnes, 2002) for selected UK lithologies

Lithology / material	Mean DOC (mg/l)	Range of f_{OC} values
Millstone Grit	3.9	n/a
Permo-Triassic Sandstone	2.8	0.00001 – 0.00071
Magnesian Limestone	2.6	n/a
Coal Measures	2.2	0.0038 – 0.073[a]
Carboniferous Limestone	2.0	n/a
Sands and gravels	1.9	0.0002 – 0.012
Chalk	1.0	0.00007 – 0.0012
Jurassic Limestone	0.84	0.0001 – 0.027[b]
Lower Cretaceous aquifers	0.62	0.0003 – 0.0019[c]

a) Lower Coal Measures only; b) Lincolnshire Limestone only; c) Lower Greensand only

Siemens *et al*. (2003), however, found that DOC leached from some agricultural soils contributed negligibly to the denitrification process because the DOC in the soils themselves appeared not to be bioaccessible. It was concluded that denitrification in the groundwater below was being controlled by the translocation of organic carbon to the soils by crop roots. Plant roots exude small organic molecules, including sugars, amino acids, organic acids and amides (Neff and Asner, 2001). These molecules influence soil nutrient availability both directly and indirectly by stimulating the activities of microbial and fungal components of the soil biota. In a comprehensive literature review, Beauchamp *et al*. (1989) found that denitrification in the presence of some of the more complex organic molecules (proteins, lipids and lignin, for example) was sometimes facilitated by bacteria performing fermentation (Figure 4.5). Fermentation is a bacterially-mediated redox process whereby organic compounds are both the electron donor and acceptor. This tends to lead to changes in the organic chemistry of the solution, as the bacteria break down complex molecules (such as sugars) to simpler ones (such as alcohols), but not to changes in the redox chemistry of the solution.

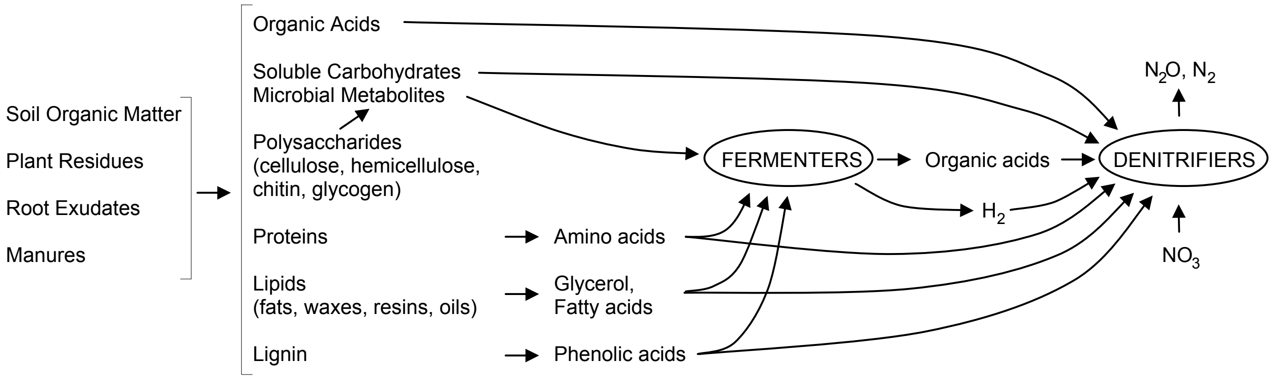


Figure 4.5. Schematic pathways of organic carbon transfer to fermenters and denitrifiers under anaerobic conditions (based on Beauchamp *et al*., 1989).

Kaiser *et al*. (2002) separated DOC into two categories: specific low-molecular-weight compounds (such as acetate) and high-molecular-weight compounds, with the former being assumed to be more biologically reactive. Similarly, Baker and Vervier (2004) found that the rate of denitrification in an alluvial aquifer was best predicted by the concentration of low molecular weight organic acids. Corre (1999) also describes how water-extractable organic carbon (WEOC) has been well correlated with denitrification capacities in different soil types. The varying availability of DOC in hydrogeological environments is discussed in Section 5. It is controlled primarily by the nature and quantity of the carbon source, but also by mineralisation (microbial oxidation to its simplest forms, such as H_2O and CO_2) and sorption to aquifer solids. Attenuation of DOC is discussed in detail in Jacinthe *et al*. (2003).

Solid-phase organic carbon contents of soil or geologic deposits, typically expressed as the solid organic matter (SOM) or fraction of organic carbon (f_{OC}), may also give some indication of the potential for denitrification. Brettar *et al*. (2002), for example, observed a positive correlation between denitrification rate and total organic carbon in a soil. SOM or f_{OC} databases, although perhaps not specifically generated to evaluate denitrification, may offer some insight. Steventon-Barnes (2002) measured numerous values of f_{OC} across common UK lithologies; apart from peat, most were very low (Table 4.2). Such f_{OC} values can only be regarded as suggestive of the relative potential for denitrification in each lithology in recognition that the nature of the carbon, its water-extractability and its bioavailability in the denitrification process will vary between and within given lithologies. Eppinger and Walraevens (2005), for example, point out that the texture (particularly the pore throat size and clay content) of a rock makes certain parts of the SOM physically inaccessible to micro-organisms, even if it is bioaccessible. For this reason a fine grained sediment, even if it is rich in SOM, may not contribute significantly more denitrification than a coarser-grained sediment.

Recent research has aimed to characterise the composition of organic carbon in sub-surface formations, where its role in influencing both sorption and biodegradation processes has been increasingly recognised (Allen-King *et al*., 2002). Looking at the type of SOM in sediments, Hartog *et al*. (2004) found that sediments with more oxidised organic matter were less reactive (via microbial action) to dissolved oxygen. The geological history of the sediments was therefore correlated with reduction potential, and sediments that had been exposed to aerobic conditions during deposition and diagenesis yielded SOM with a lower reactivity. Postma *et al*. (1991) and Kölle *et al*. (1993) both noted that denitrification by reaction with organic carbon present as lignite or coal fragments in a sediment was minimal.

Denitrification not only consumes natural organic carbon, but the ubiquitous presence of denitrifying bacteria in the sub-surface can contribute to attenuation of the impacts of organic contaminants. It is beyond the scope of this report to discuss denitrification in this context, but the following publications provide useful introductions and reference lists and are indicative of the wide range of organic contaminants to which denitrification is relevant.

Benzene, toluene, ethylbenzene and xylenes (BTEX) components from petroleum are often degradable under denitrifying conditions (Morgan *et al*., 1993; Rabus and Widdel, 1996). Denitrification with benzene as the electron donor is not always observed in practice (Johnson *et al*., 2003), in part because of the sporadic distribution of the bacteria able to bring about this reaction (Kao and Borden, 1997). In the context of remediation, Eckert and Appelo (2002) used an injection of potassium nitrate to enhance oxidation of BTEX compounds in a sandy aquifer.

A variety of other petroleum hydrocarbons, including aliphatic and aromatic hydrocarbons, have been shown to be subject to degradation, albeit often slowly, under denitrifying conditions (Bregnard *et al.*, 1997; MacRae *et al.*, 1998).

Phenols, cresols and related compounds can all be biologically degraded by denitrifying bacteria (Broholm and Arvin, 2000). However, at elevated contaminant concentrations, degradation is likely to be inhibited (Spence *et al*., 2001).

Ethanol was used in the bioremediation of nitrate contaminated groundwater by Tartakovsky *et al*. (2002) and Bates and Spalding (1998). Natural attenuation was initially not effective because of aerobic conditions in the aquifer, but the addition of ethanol depleted the dissolved oxygen and provided an electron donor for the denitrification reaction.

Chlorinated solvents such as tetrachloromethane (carbon tetrachloride) are subject to biodegradation under denitrifying conditions and nitrate addition has been applied to enhance bioremediation processes (Dybas *et al*., 1998). Lower-chlorinated solvents and related compounds such as dichloroethene and vinyl chloride are also reported to be biodegraded under denitrifying conditions (Rijnaarts *et al*., 1997) but the process has been little studied and does not bring about degradation of more highly chlorinated solvents such as trichloroethene.

For landfill leachates, Christensen *et al*. (2000) discuss denitrification in the context of redox zonation within landfill plumes. There is usually sufficient organic carbon within landfill leachate to take the redox condition to methanogenesis. Nitrogen in the plume is present as ammonium; nitrate is not formed where anaerobic conditions exist, so its reduction only occurs at the outer halo of the migrating plume, where surrounding groundwater nitrate mixes with organic carbon and electron donors in the plume.

For sewage effluents, a number of studies (including Robertson *et al*., 1991; Wilhelm *et al*., 1994; MacQuarrie *et al*., 2001a) found that although raw sewage contains considerable amounts of labile organic carbon, much is oxidised coincidentally with, or prior to, ammonium oxidation in the unsaturated zone. Nitrate-rich, well-oxidised effluent may therefore contain only small amounts of carbon that might act as an electron donor. Furthermore, this is likely to be the least bioavailable fraction of the original organic carbon load. Any denitrification in these systems therefore has to largely rely on the presence of in situ electron donors. For example, DeSimone and Howes (1998) found that organic compounds in a waste water plume were completely mineralised before denitrification started and the in situ electron donors in the sandy host aquifer could only contribute a two per cent decrease in the nitrogen load. On the other hand, Spalding *et al*. (1993) studied a plume arising from sewage sludge disposal that did contain sufficient labile bioavailable organic carbon to be used for denitrification.

4.4.2 Reduced iron (autotrophic denitrification)

There is some evidence that groundwaters containing Fe^{2+} may normally contain little or no nitrate (Korom, 1992). Reduction of nitrate by Fe^{2+} can be either abiotic, biotic, or a combination of both. The abiotic reduction process is not well understood. Davidson *et al.* (2003) demonstrate that Fe^{2+} acts to promote abiotic denitrification, in which the Fe^{2+} reduces nitrate to nitrite, then is regenerated by the oxidation of organic carbon. Alternatively, the Fe^{3+} can precipitate as an oxyhydroxide or oxide mineral. Nitrite can then be then abiotically reduced in an organic-poor environment to gaseous nitrogen compounds by the further oxidation of iron (Korom, 1992). Some stoichiometric equations for the overall process are presented in Ottley *et al*. (1997):

$$\begin{aligned}
8Fe^{2+} + NO_3^- + 13H_2O &\rightarrow 8FeOOH + NH_4^+ + 14H^+ \\
8Fe^{2+} + 2NO_3^- + 11H_2O &\rightarrow 8FeOOH + N_2O + 14H^+ \\
10Fe^{2+} + 2NO_3^- + 14H_2O &\rightarrow 10FeOOH + N_2 + 18H^+ \\
2Fe^{2+} + NO_3^- + 3H_2O &\rightarrow 2FeOOH + NO_2^- + 4H^+ \\
12Fe^{2+} + NO_3^- + 13H_2O &\rightarrow 4Fe_3O_4 + NH_4^+ + 22H^+ \\
12Fe^{2+} + 2NO_3^- + 11H_2O &\rightarrow 4Fe_3O_4 + N_2O + 22H^+ \\
15Fe^{2+} + NO_3^- + 13H_2O &\rightarrow 5Fe_3O_4 + N_2 + 28H^+ \\
12Fe^{2+} + 2NO_3^- + 13H_2O &\rightarrow 4Fe_3O_4 + NH_4^+ + 22H^+
\end{aligned}$$

The biotic process of nitrate reduction by Fe^{2+} has been identified in a number of studies (Korom, 1992, and refs therein) and is due to the ubiquitous bacterium *Gallionella ferruginea. G. ferruginea* is known to autotrophically reduce NO_3^- to NO_2^- in a reduced iron environment, but does require a small amount of oxygen for growth, so a likely ecological niche is at an aerobic/anaerobic interface where Fe^{2+} and dissolved oxygen meet in opposing diffusion gradients.

Copper (II) appears to play an essential catalytic role in the abiotic reduction process and it is the solid phase that has the catalytic role (Ottley *et al*., 1997). However, it is the concentration of copper (II) in solution that controls the rate of reaction, and at typical UK groundwater concentrations of 0.4 to 3.2 μg/l the rate of reaction would be appreciable. Solid phase forms of Ag(I), Cd(II), Ni(II), Hg(II) and Pb(II) also catalyse the reduction reaction, with a slight effect noted in the presence of Mn(II). There is also evidence for slow abiotic reduction of nitrate in the absence of added metal catalysts.

Because of the direct role played by protons in the reactions, the reaction rate is pH-dependent, with the rate increasing with increasing pH (Ottley *et al*., 1997). Under neutral or alkaline conditions the Fe^{3+} is precipitated as ferric oxide or oxyhydroxide. This precipitation reaction releases H^+ ions into solution, balancing some of their consumption by the denitrification reactions. Mn^{2+} and HS^- are also potential electron donors for autotrophic or abiotic denitrification reactions.

Sources of dissolved ferrous iron in aquifers include the oxidation of iron sulphide and the dissolution of some silicate minerals such as biotite, pyroxenes and amphiboles. Iron sulphide minerals tend to occur in sediments that were deposited under anaerobic conditions. These are commonly clayey or contain much organic carbon. Typical UK lithologies that contain significant amounts of iron sulphide include the coal measures, some units in Carboniferous limestone, the Oxford and Kimmeridge clays, plus many of the hard rock formations of Scotland, Cumbria, Wales and south west England, which contain hydrothermal iron sulphides. Glacial tills derived from the weathering of these strata also contain significant amounts of iron sulphide. Ferrous iron-rich silicates derive ultimately from the more alkaline (‘basic’) igneous and metamorphic rocks, and again tend to be found in the harder rocks of the north and west UK. An exception is glauconite, which contains Fe^{2+} and is found especially in the Greensand formations in south east England, but is present in smaller quantities in other Jurassic, Cretaceous and Tertiary shallow marine sediments.

4.4.3 Reduced sulphur (autotrophic denitrification)

Electrons needed for denitrification can also originate from the microbial oxidation of reduced sulphur to the S(+VI) state as sulphate. The reduced sulphur may be present as the S(-II) state in H_2S, S(-I) in FeS_2, S(0) in elemental sulphur, S(+II) in thiosulphate $S_2O_3^{2-}$, or S(+IV) in sulphide SO_3^{2-}. Under typical aquifer conditions, iron (and sometimes manganese) sulphide can be utilised as the electron donor (Korom, 1992):

$$5FeS_2 + 14NO_3^- + 4H^+ \rightarrow 7N_2 + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O$$

This reaction is mediated by all manner of autotrophs and heterotrophs, including *Thiobacillus denitrificans*, which is recognised as the archetypal organism undertaking this process. Oxidation of sulphur therefore provides a viable alternative electron donor in carbon-limited systems (Moncaster *et al*., 2000; Kelly, 1997; Robertson *et al*., 1996; Kölle *et al*., 1985; Tesoriero *et al*., 2000; Broers, 2005). The susceptibility of pyrite to oxidation depends on its microscopic structure; thus, not all the pyrite in a sediment may be available (Kölle *et al*., 1985).

Autotrophic denitrification by sulphide can, however, be detrimental to well-field operations. Although nitrate loading may be decreased, the following issues potentially remain (Kölle *et al*., 1985; van Beek and van Puffelen, 1987):

- Increasing sulphate concentrations, where stoichiometrically, a decrease of 10 mg N/l can lead to an increase of approximately 50 mg SO_4/l. In a high sulphate groundwater, this may increase the sulphate concentration above the drinking water standard (250 mg SO_4/l). In addition the hardness of the water increases, as does its corrosion potential.

- Well performance can be diminished by ferric iron precipitation at the well screen. Increased concentrations of dissolved iron and manganese lead to increasing water treatment costs.

Release of heavy metals as a by-product of pyrite oxidation is a well-known phenomenon in the context of mine-water chemistry (Bowell, 2002). Although no studies appear to address this in the context of denitrification, there is no reason to believe that it does not also occur to some degree. However, the increase in pH associated with the denitrification reaction may quickly render these metals immobile.

In many anaerobic aquifers, a sulphate-reducing zone lies down the flow line from the nitrate-reducing zone (Christensen *et al*., 2000 and references therein). Hence, sulphate arising from denitrification reactions may be transformed back to sulphide, if organic carbon is available as an electron donor in the sulphate-reducing zone. Korom (1992) describes how at one site the denitrification reaction had a first-order half-life of 1.2 to 2.1 years, while the sulphate reduction reaction downgradient had a half-life of 76 to 100 years, leading to the development of a distinct sulphate plume.

4.4.4 Multiple electron donors

Denitrification reactions in any given region or aquifer may not all be driven by one electron donor because in certain situations, both organic carbon and sulphide minerals may be available. This is a complex subject, but it does appear that denitrification by multiple electron donors can occur in the same system. At the simplest level, this may be due to a change in lithology along a flow path: for example, Böhlke *et al*. (2002) identified three different environments of denitrification along the flow paths in a superficial sand aquifer. The recharge zone of the sandy aquifer contained both iron sulphides and organic carbon; in this environment, only the iron sulphide was being oxidised. At the discharge zone to a riparian wetland, iron sulphide acted as the electron donor at depth, but organic carbon was reduced at shallow depths where it was more abundant. Aravena and Robertson (1998), however, identified a plume in which it seems that although sulphide was the primary electron donor, there was also significant oxidation by organic carbon in approximately the same region of aquifer. Postma *et al*. (1991) also identified a sand and gravel aquifer containing both organic carbon and pyrite, both of which contributed to denitrification. However, reduction by pyrite was the dominant denitrification process because in this aquifer, the organic carbon appeared to be poorly bioavailable.

Since the sulphide-oxidising denitrification reactions releases Fe^{2+}, reduced iron may also contribute to the denitrification potential (Kölle *et al*., 1985).

4.5 Environmental conditions

4.5.1 Effect of nitrate concentration

Some references (such as Morris *et al*., 1988; Smith and Duff, 1988; Korom *et al.* 2005) indicate that the kinetics of denitrification at concentrations greater than 1 mg N/l are zero order (that is, independent of concentration). However, Korom (1992) questions this and suggests that the rates may show concentration dependence, but caveats this with the statement that there is insufficient data in the studies to provide a definitive opinion.

Excess concentrations of nitrate affect the denitrification process by inhibiting the formation of N_2 gas; in such cases, the denitrification chain ends with the release of N_2O (Blackmer and Bremner, 1978). These concentrations are case specific, but in some cases even low concentrations (relative to typical groundwater conditions) affect the ratio of $N_2O:N_2$ evolved. Magalhàes *et al.* (2003), for example, show an increase in that ratio from 0.11 to 0.34 between an addition of 0 mg/l as N to 4 mg/l as N, coupled with a decrease in the denitrification efficiency.

The relative concentrations of nitrate and organic carbon appear to control whether nitrate is depleted by denitrification or dissimilatory nitrate reduction to ammonium. This is discussed in more detail in the description of the latter process (Section 4.9.1).

4.5.2 Effect of oxygen concentration

The denitrification process is thermodynamically less favourable than the reduction of dissolved oxygen. In a system that contains oxygen, nitrate and organic carbon, the oxygen will normally be the preferred electron acceptor over nitrate; denitrification will therefore be principally an anaerobic process. Numerous examples illustrate how denitrification only starts when dissolved oxygen levels reach a certain low threshold. Table 4.3 lists dissolved oxygen concentrations below which denitrification has been observed to take place in the field. There appears to be no consensus, but it seems reasonable to assume that, given all other prerequisites, denitrification will probably occur at concentrations below 1 mg/l and may even occur at concentrations below 2 mg/l. In those cases where it was quantified (such as DeSimone and Howes, 1998), denitrification rates tended to be higher in regions of the lowest oxygen concentrations.

Micro-organisms in soil or sediment do not necessarily ‘experience’ the same concentrations as those measure by a dissolved oxygen probe in a mixed sample. While a water sample from a piezometer may be measured in tens or hundreds of millilitres, the amount of water surrounding a 1 μm diameter microbe will be measured in the scale of 10^{-9} millilitres. There therefore needs to be only a very small volume of water, relatively isolated from mixing with the bulk oxygenated groundwater, within which denitrifying bacteria can begin to use nitrate. The threshold concentrations in Table 4.3 are thus only a guide to the conditions under which denitrification can occur, where soil texture may play an important role in determining them.

Table 4.3. Approximate dissolved oxygen concentrations in groundwater below which denitrification has been observed

Dissolved oxygen concentration (mg/l O_2)	Conditions*	Reference
4	Agricultural fertiliser plume	Böhlke and Denver, 1995
2-3	Agricultural fertiliser plume	Tang and Sakura, 2005
2	Literature survey	Bates and Spalding, 1998
2	Septic waste plume	Gillham, 1991
1.2	Agricultural fertiliser plume	Gallardo and Tase, 2005
1	Agricultural fertiliser plume	Puckett and Cowdery, 2002
1	Agricultural fertiliser plume	Böhlke *et al*., 2002
1	Landfill plumes	Christensen *et al*., 2000
1	Natural (arid zone so T~30ºC)	Vogel *et al*., 1981
1	Septic waste plume	DeSimone and Howes, 1998
1	Septic waste plume	Starr and Gillham, 1993
0.2	Tracer injection experiment	Trudell *et al*., 1986

* groundwater temperatures are seldom presented

Carter *et al*. (1995) managed to isolate several species of bacteria from the soil environment that are capable of denitrification under aerobic conditions (at up to 80 per cent air saturation), and respire oxygen and nitrate simultaneously. Aerobic denitrifying fungi also occur (Cannavo *et al*., 2004 and references therein). However, examples of aerobic denitrification in the groundwater environment are rare to non-existent. In studies in which aerobic denitrification has been postulated, on closer investigation denitrification seems to occur under locally anaerobic conditions within micro-sites in particulate organic matter (Hammersley and Howes, 2002) or in heterogeneous organic-rich patches of sediments (Jacinthe *et al*., 1998) or within biofilms (Seiler and Vomberg, 2005). The presence of such micro-anaerobic environments could explain why there are some unexpectedly high dissolved oxygen concentrations in Table 4.3.

One consequence of denitrification under aerobic conditions is that the presence of molecular oxygen tends to arrest the formation of N_2O. For example, Jacinthe *et al.* (1998) observed denitrification in soils at a dissolved oxygen concentration of 2.2 mg O_2/l (at the experimental temperature of 11ºC; 21 per cent air saturation). The rate of denitrification was low but measurable. However, there was very little generation of nitrogen gas as a reaction product: 90 per cent of the gas produced in the column was N_2O.

4.5.3 Effect of nutrient and micro-nutrient availability

Denitrifying bacteria obtain their energy for metabolism and growth from the oxidation of organic carbon, sulphide minerals or reduced species of iron and manganese. Bacteria also require carbon and other nutrients for construction of their cellular structure (Table 4.4), and micronutrients (such as B, Cu, Fe, Mn, Mo, Zn and Cl) for cell construction, production of enzymes, energy transfer and other processes.

Table 4.4. Atomic composition of a typical bacterium (Keddy, 2000)

Element	C	H	N	O	P	S	Total
Percent mass	12.1	9.9	3.0	73.7	0.6	0.3	99.6

Although most groundwaters contain adequate concentrations of the necessary minerals to support microbial growth (Champ *et al*., 1979), systems where nutrients or micronutrients are absent or present only in small quantities may limit the extent of bacterial growth and hence denitrification. The availability of phosphorus may be a key limiting factor in some systems. Hunter (2003) studied denitrification in sand columns using a phosphate-limited eluent and found that only a small amount of nitrate was removed as nitrogen, where most was converted to nitrite. 0.16 mg P/l was required to effectively remove 17 mg N/l nitrate without significant accumulation of nitrite (a molar ratio of 235 N:P).

The mobility of phosphate is controlled by sorption to mineral surfaces (Tofflemire and Chen, 1977), along with the solubility of calcium phosphates in alkaline environments, and iron and aluminium hydroxyl phosphate minerals in acid environments (Brady and Weil, 2002). For example, Harman *et al*. (1996) found that attenuation of phosphate in a plume from a septic system in a sand aquifer was by precipitation of minerals (300-500 mg P/kg) in the unsaturated zone and sorption (<11 mg P/kg) in the groundwater zone. Robertson *et al*. (1998) studied plumes from septic systems in nine sandy aquifers and a sandy till. The highest phosphate concentrations occurred under oxidising conditions at near-neutral pH. Sorption controlled mobility in these aquifers, with retardation factors of 20 to 100; the breakthrough of elevated phosphorus concentrations was thus significantly delayed behind that of nitrogen concentrations. Nitrite has been found to accumulate in a phosphate-limited system (Hunter, 2003).

The presence of sulphur as sulphate and thiosulphate have been shown to inhibit denitrification in soils, with the rate of denitrification negatively correlated to the sulphate (or thiosulphate) concentration (Kowalenko, 1979). In soil, sulphide has been shown to promote dissimilatory reduction of nitrate to ammonium rather than denitrification (Hiscock *et al*., 1991), although Beauchamp *et al*. (1989) cites examples where the presence of sulphide alleviates the acetylene blockage of the conversion from N_2O to N_2.

4.5.4 Effect of pH

The pH range preferred by heterotrophic soil micro-organisms (denitrifying bacteria that use organic carbon as the electron donor) is generally considered to be between 5.5 and 8.0 (Rust *et al*., 2000). pH values outside this range may negatively impact upon the denitrification process, but the optimal pH is site-specific because of the effects of acclimation on the microbial ecosystem. Concentrations of dissolved phosphate, for example, are controlled by pH and are highest at near-neutral pH values (Robertson *et al*., 1998). The rate of autotrophic denitrification by reaction with Fe^{2+} is also controlled by pH (Section 4.4.2).

Strongly acidic environments (pH<5) inhibit rapid denitrification and tend to arrest the denitrification chain with the formation of nitrite or N_2O (Brady and Weil, 2002). Low pH groundwater can arise, for example, where organic wastes are oxidised to organic acids. This need not be an issue in well-buffered calcareous aquifers (Robertson *et al*., 1998; Amirbahman *et al*., 1998), but in non-calcareous aquifers the mineralisation of organic carbon and nitrification of ammonium in organic wastes can reach pH 4.9 (DeSimone and Howes, 1998; Wilhelm *et al*., 1996) and possibly below. However, abiotic denitrification has nevertheless been observed in very low pH (<4.5) soils (references cited in Beauchamp *et al*., 1989).

Denitrification itself can increase the pH of the surrounding solution by releasing CO_2 and OH^-. Normally these combine to yield HCO_3^- but if the production of hydroxyl ions exceeds that of carbon dioxide, the pH can rise. Rust *et al.* (2000) quote an acceptable upper limit for pH of 8.3, above which denitrification is arrested.

4.5.5 Effect of temperature

Optimum temperatures for denitrification are 25 to 35ºC but denitrification processes will normally occur between two and 50ºC (Brady and Weil, 2002) and may occur beyond these limits where bacteria have evolved to cope with specific environmental conditions. It is often assumed that the reaction rate doubles for every 10ºC increase in temperature. Denitrification can therefore be expected to take place reliably at typical UK groundwater temperatures (usually around 11°C). As would be expected, Lind (1983) found that denitrification at in situ (10ºC) temperatures was significantly less than at lab temperatures (25ºC), but that rate increases varied considerably for different soil samples, from 1.7 times to 23 times over the temperature range.

In the IMPACT model of Andrews *et al*. (1997b) the rate of denitrification in the soil zone was described by the following first-order temperature-dependant relationship, based on iterative modelling and sensitivity studies of the field sites:

$$\frac{\partial(N_2)}{\partial t} = \kappa_{dn} \times 1.07^{(T_{30} - 21)} \times (NO_3 - N)$$

where nitrogen concentrations are measured in mass units
t is time (days)
κ_{dn} is the rate constant for denitrification at 21ºC (0.1 day^{-1} in the model)
T_{30} is the temperature of the soil at 30 cm depth (ºC)

It is difficult to observe temperature dependency of denitrification rates in the relatively stable groundwater environment, but Saunders and Kalff (2001) and Grischek *et al.* (1998) observed this dependency in the hyporheic zone of a lake and a river respectively. Saunders and Kalff (2001) found that an increase of about 5°C resulted in a ten-fold increase in denitrification rate. Robertson *et al*. (2000) show that there is a correlation between water temperature and denitrification rates in a permeable reactive barrier system in Canada. Denitrification rates were observed even down to 2ºC: between 2 to 5ºC, rates were approximately 5 mg N/l/day. Between 10 to 20ºC, rates increased to 15 to 30 mg N/l/day.

Christiansen and Cho (1983) report that abiotic denitrification of nitrite by soluble organic matter can occur in frozen soil. At one field site, Cannavo *et al*. (2004) observed that unlike CO_2 levels, N_2O levels in soil were independent of temperature; the authors suggested that this was due to the action of aerobic denitrifying fungi which were much more tolerant of low temperatures than bacteria.

Changes in the rate of denitrification with seasonal temperature variations may be masked by variations in the rate of organic carbon flux. For example, Cannavo *et al*. (2004) found that freeze-thaw cycles increase the flux of carbon to the unsaturated zone and can create anaerobic micro-environments in the soil in which denitrification can become established.

4.5.6 Effect of salinity

High salinities (such as in wastewaters) are known to inhibit, but not necessarily completely arrest, denitrification. Dinçer and Kargi (1999) show that denitrification is inhibited by concentrations of salt greater than 20 000 mg/l NaCl (57% sea water), while Ukisik and Henze (2004) found that denitrification rates were reduced to 10% between 4800 and 97 000 mg/l Cl (22% - 440% sea water). However, in estuarine and marine environments, denitrification rates do not appear to be affected by the salinity in which they occur (Magalhàes *et al.*, 2003; Kana *et al.*, 1998; Granl, 1999) and appear to be halo-tolerant strains.

4.5.7 Effect of toxins

Heavy metals, pesticides and pesticide derivatives are known to inhibit denitrification (references cited in Hunter, 2003; Sáez *et al*., 2003). In contrast, Sims (1990) points to a number of instances where pesticides have no effect on, or even stimulate, denitrification. More recently, Jørgensen *et al.* (2004) found that in a column of till that was depleted in natural DOC, denitrification rates were low until a pulse of pesticides (bentazon, MCPA, MCPP, fenoprop and propoinol) was added that acted as a source of organic carbon.

4.5.8 Effect of sediment pore size

Intergranular flow provides a high surface area to volume ratio for microbial growth and the pore spaces represent the regions of greatest biomass and metabolic activity (for example, data for the Chalk and Sherwood Sandstone in Blakey and Towler 1988; Environment Agency, 1999). The exception to this is when pore spaces are too small to permit microbial growth.

For example, Rees (1981) noted the absence of microbial activity of any kind in the pore spaces of unfissured Lower Chalk underlying a landfill in Oxfordshire. Whitelaw and Rees (1980) confirmed the presence of denitrifying bacteria in the unsaturated zone, to a depth of at least 50 m, of the Middle and Upper Chalk underlying agricultural land but proposed that microbial activity was confined to fissures (Section 5.1.4). Both papers concluded that penetration of microbial cells (typical diameter = 1 µm) was precluded by the small pore sizes of the Chalk (median diameter = 0.22 µm for the Lower Chalk and 0.5-0.7 µm for the Middle and Upper Chalk; Rees 1981). Certainly, a large microbial population could not develop in the pore space of the Chalk, which implies negligible denitrification in poorly fissured Chalk aquifers. The same can be said for the fine-grained Jurassic Lincolnshire limestone (Bottrell *et al.,* 2000), although Lawrence and Foster (1986) did provide evidence of denitrification occurring within the matrix immediately adjacent to fissures, perhaps because the matrix was more open in the weathered zone adjacent to the fractures (Section 5.2.2).

In general, the pore sizes of other UK aquifers are larger (British Geological Survey and Environment Agency, 1997, 2000) and therefore, all other factors being equal, more likely to support an active microbial population (Environment Agency, 2001).

Conversely, where large fractures represent the predominant flow pathway in a formation, there will be a small surface area for microbial growth relative to the fracture volume, and a comparatively short hydraulic residence time within the fractures. Consequently, the rate of biodegradation activity in a fracture flow system will be low compared to an intergranular system (Mather, 1989) but no published information appears to have explicitly considered the rates of denitrification. However, the reduced biodegradation potential of organic contaminants during fracture flow is well-known (for example, Wealthall *et al.,* 2001) and denitrification rates can be expected to be significantly less in such systems.

4.6 Microbial acclimation

Acclimation is the requirement for a 'lead time' before a microbial population can adapt to using a new substrate. There is much evidence that denitrifying bacteria are ubiquitous in the natural environment and can respond quickly to nitrate inputs, provided that other environmental conditions are conducive to their activity. For example, Casey and Klaine (2001) and Casey *et al*. (2001) investigated whether a riparian wetland could support a population of denitrifying bacteria when nitrate inputs were from infrequent storm pulses only. Within the site, denitrification rates were higher at the upgradient edge, where nitrate exposure was more frequent. It was concluded that a population of denitrifying bacteria was maintained between inflow pulses, but denitrifying enzyme activity appeared to improve with increased previous exposure to nitrates and required a 'priming' period of several hours for populations to be re-established on exposure.

Cannavo *et al.* (2004) found a similar pattern of denitrifying potential down through an unsaturated zone beneath a maize field. While the rate of denitrification was controlled by the supply of organic carbon in the upper layers (<1m), at greater depths the limiting factor was exposure to nitrate. The authors suggested that the denitrifying community at depth was less likely to have synthesised denitrifying enzymes, but this was not demonstrated.

4.7 Lines of evidence for denitrification

4.7.1 Stable isotope fractionation

The stable isotope composition of nitrate is known to be indicative of its source and can also be used to indicate that biological denitrification is occurring. The variable used is δ^{15}N which compares the fraction of ^{15}N/^{14}N of the sample to that of an internationally accepted standard (the air in the case of nitrogen):

$$\delta^{15}\mathrm{N}\,(‰) = \frac{\left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right)_{\mathrm{sample}} - \left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right)_{\mathrm{s\,tan\,dard}}}{\left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right)_{\mathrm{s\,tan\,dard}}} \times 1000$$

When tracing the origins of contamination, some sources have characteristic isotopic signatures. For instance, the δ^{15}N values for inorganic nitrate fertilisers tend to be in the range –7 to +5 ‰, for ammonium fertilisers –16 to –6 ‰, for natural soil –3 to +8 ‰, and for sewage, +7 to +25 ‰ (Fukada *et al.,* 2004; Widory *et al*. 2004; BGS, 1999). For example, in groundwater samples from the East Midlands Sherwood Sandstone aquifer, Wilson *et al.* (1994) identified influences of animal waste (+22.7 to +31.1 ‰), naturally nitrified soil organic nitrogen (+5.4 to +9.3 ‰), nitrate-based fertilisers (-1.3 to+4.5 ‰) and ammonium-based fertilisers (-15.7 to -5.8 ‰). This approach is often combined with information from other species of interest: Barrett *et al.* (1999) used δ^{15}N and microbiological indicators to identify sewage N, while Widory *et al*. (2004) used δ^{15}N, δ^{11}B and ^{87}Sr/^{86}Sr to discriminate between mineral fertilisers, sewage, and pig, cattle and poultry manure. Bölke and Denver (1995) use δ^{15}N with δ^{13}C, δ^{34}S, chloroflurocarbons, tritium and major ion chemistry to determine the application history and fate of nitrate contamination in agricultural catchments.

Biological processes such as denitrification selectively use molecules containing lighter isotopes of the constituent atoms, where bonds with such atoms are slightly weaker, and it is therefore thermodynamically favourable to get the same amount of energy from a reaction by breaking a weaker bond. Consequently, as these processes proceed, there is a trend towards the reacting molecule being enriched in the heavier isotope. As the ratio $^{15}N/^{14}N$ increases with reaction, $\delta^{15}N$ tends towards more positive values and changes in $\delta^{15}N$ along flow lines indicates that a biological transformation is occurring.

The same fractionation process also applies to the oxygen isotope measurement $\delta^{18}O$. It is used less frequently because the analysis of oxygen isotopes in the nitrate ion is analytically difficult. Based on an evaluation of microbial denitrification in a sandy aquifer, however, Bötcher *et al*. (1990) concluded that the $\delta^{18}O$ of nitrate was the most reliable indicator of the denitrification process because it is less variable in the original source. However, Fukada *et al.* (2003 and 2004) argue that both isotopic ratios are of value because the effects of denitrification project along a straight line on a $\delta^{15}N$ - $\delta^{18}O$ cross-plot. The gradient of the line reflects the ratio of preferential enrichment of heavy isotopes of N and O; a value of $\delta^{15}N/\delta^{18}O = 1.3$ to 2.1 appears to be characteristic of the denitrification reaction.

Use of isotopic ratios is becoming a standard technique to identify the occurrence of denitrification, and is used in many papers listed in this study. These include studies of denitrification in aquifers (Sections 5.2 to 5.3; Griggs *et al.,* 2003; BGS, 1999; Spalding *et al*., 1993; Starr and Gillham, 1993; Smith *et al*., 1991; Vogel *et al*., 1981; Widory *et al.* 2004), riparian zones and the hyporheic zone (Section 5.5; Clément *et al.,* 2003; Devito *et al*., 2000; Mengis *et al*., 1999; McMahon and Bölke, 1996), and permeable reactive barriers (Section 5.6; Robertson *et al*., 2000).

4.7.2 Nitrogen-argon ratio (‘excess nitrogen’)

Water that has equilibrated with the atmosphere will have a dissolved N_2/Ar ratio of 37-39 (ml/l:ml/l) (Wilson *et al.*, 1990), the actual value being dependant on temperature. As the waters percolate through the unsaturated zone, the increase in pressure forces trapped bubbles into solution, known as ‘extra air’ (Vogel *et al.*, 1981). Since air has a N_2/Ar ratio of 83.5, the ratio in solution increases by a few percent; this can be quantified by measuring dissolved concentrations of neon (Wilson *et al.*, 1994).

Denitrification adds only N_2 to solution in addition to the extra air, and the degree of denitrification can be computed by measuring the departure of a N_2 concentration data point from the extra air mixing line – called the ‘excess nitrogen’ (Figure 4.6). For example, Wilson *et al.* (1990) identified the following sequence of N_2/Ar ratios down-dip through the Lincolnshire limestone:

Atmospheric equilibrium: $N_2/Ar = 38$ → *Extra air entrainment:* $N_2/Ar = 41$ →
Denitrification: $N_2/Ar = 42 – 55$

Here denitrification accounts for up to 25 per cent of the total dissolved N_2. The N_2/Ar ratio of 55 at one site corresponds to the reduction of 7.5 mg N/l.

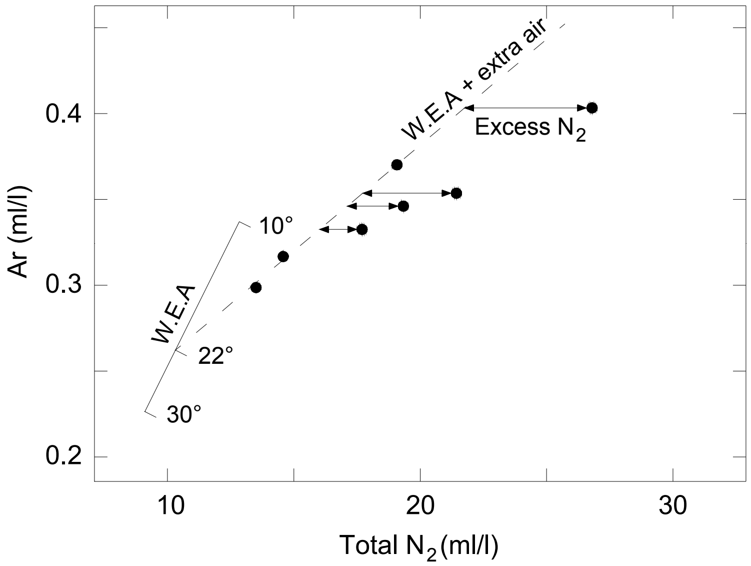


Figure 4.6. Graph illustrating the derivation of excess nitrogen to identify denitrification. WEA is water in equilibrium with atmosphere (10ºC to 30ºC). Excess N_2 is calculated as the difference of the measured N2 concentration from the 'WEA plus extra air' line (after Vogel *et al.*, 1981).

4.7.3 Hydrochemical parameters

Apart from changes in concentrations of redox species, a very simple technique for identifying potential denitrification is to compute the change in the Cl^-/NO_3 ratio. Because neither chloride nor nitrate are affected by chemical processes in groundwater (except where NO_3 may undergo denitrification), this compensates for changes in nitrate concentration caused by mixing of groundwaters with different composition.

4.8 Denitrification kinetics and modelling

The most common approach applied for modelling denitrification in the sub-surface has been the use of Monod kinetics. These are applied to systems where the rate of cell growth is controlled by the availability of a growth substrate (nitrate in the following examples), and biomass increase takes place as a result of the biodegradation of the growth substrate (Environment Agency, 2002). The kinetics can be represented mathematically by:

$$\mu = \mu_{max} \frac{S}{K_S + S}$$

where μ = the specific growth rate of the denitrifying micro-organism (day^{-1})
μ_{max} = the maximum growth rate of the denitrifying micro-organism (day^{-1})
S = the substrate concentration (mg/l),
K_S = the half-saturation constant (the substrate concentration at which the growth rate is half the maximum growth rate; mg/l)

This relationship can be expanded to compute reaction rates for cases in which there are multiple aqueous reactants and multiple reaction steps, such as the full description of denitrification (for instance, MacQuarrie and Sudicky, 2001). These can be then used to compute the speciation of nitrogen compounds (for example, between NH_3, NH_4^+, N_2, NO_3^- and NO_2^-) in freshwater based on kinetics (Chetboun and Bachmat, 1981).

When the substrate concentration is low, ($S << K_S$), the previous equation simplifies to:

$$\mu = \mu_{max} \frac{S}{K_S}, \qquad \text{therefore:} \qquad \mu = \lambda S$$

where λ = the first order reaction rate coefficient (day^{-1}).

Sheibley *et al*. (2003) note that environmental nitrate concentrations will often be significantly below the reported range of K_S values for nitrate (0.2 to 170 mg N/l) and therefore that modelling can reasonably be based on first order kinetics in these cases. This has also been found to be the case by Jørgensen *et al*. (2004), McGuire *et al*. (2002) and Fujiwara *et al*. (2002), among others. The rate of first order reactions is often described using a reaction half-life (in days); the rate constant, λ, can be converted to a half-life with:

$$t_{1/2} = \ln(2) / \lambda$$

When the substrate concentration is high ($S >> K_S$), Equation 1 simplifies to:

$$\mu = \mu_{max}$$

and the reaction behaves like a zero-order reaction, where the rate is independent of concentration. Denitrification that can be described with zero-order kinetics has also been observed by Van Beek and Van Puffelen (1987), Starr and Gillham (1989) and Trudell *et al*. (1986) among others. Zero-order reactions seem to be identified more often in field studies than laboratory studies, but perhaps reflect a lack of data (or data scatter) in the field.

Some workers have also used empirical models of denitrification for specific circumstances. For example, Andrews *et al.* (1997a, b) used an empirical model to evaluate the significance of denitrification on the cycling and leaching of nitrogen arising from sewage sludge and fertiliser applications to soils overlying chalk.

Where reaction rates are fast relative to groundwater movement, equilibrium modelling may also be used to predict the movement of nitrate plumes or redox fronts. For example, Postma *et al*. (1991) successfully used PHREEQM [now available as PHREEQC-2 (Pankhurst and Appelo, 1999)] to predict the vertical movement of a redoxcline through a sand and gravel aquifer containing pyrite and organic carbon, where the rate of movement was controlled by the concentration and distribution in the sediments. Beller *et al.* (2004) also used PHREEQC to simulate movement and transformation of nitrate in an aquifer where pyrite oxidation alone was responsible for the denitrification.

Wriedt *et al.* (2005) developed a reaction model within MODFLOW and RT3D (Clement, 1997) to predict denitrification rates in a shallow aquifer. Nitrate inputs were distributed according to land-use, while concentrations of pyrite and sedimentary organic matter were distributed based on overlying soil types. This model provides a promising approach but is unfortunately untested.

4.9 Nitrate depletion mechanisms other than denitrification

4.9.1 Dissimilatory nitrate reduction to ammonium

Dissimilatory nitrate reduction to ammonium (DNRA) is a further anaerobic reduction reaction that can be used by fermentative bacteria (Korom, 1992). Robertson *et al.* (1996) present the following stoichiometric equation:

$$2H^+ + NO_3^- + 2CH_2O \rightarrow NH_4^+ + 2CO_2 + H_2O$$

The DNRA reaction occurs under much the same conditions as denitrification but is less commonly observed in practice. The partitioning of nitrate between denitrification and DNRA is believed to be controlled by the availability of organic matter: DNRA is the favoured process when nitrate (electron acceptor) supplies are limiting, and denitrification is favoured when carbon (electron donor) supplies are limiting (Korom, 1992; Kelso *et al.,* 1997). One important distinction between denitrification and DNRA is that the fermentative bacteria that carry out DNRA are obligate anaerobes (Hill, 1996), so cannot occupy all the niches that denitrifiers can, particularly in soil or the unsaturated zone.

Once the ammonium or nitrite generated by DNRA is released back into an aerobic environment, it will quickly be oxidised back to nitrate or taken up by vegetation. However, sorption and ion exchange of ammonium (Environment Agency, 2003) and nitrite (Davidson *et al*., 2003) is significant in many aquifer systems, so DNRA may provide a mechanism for the apparent attenuation of nitrate.

DNRA is rarely thought to be the dominant nitrate reduction mechanism in groundwater systems, but Bulger *et al*. (1989) observed DNRA of nitrate in groundwater flowing beneath waste stabilisation ponds discharging organic-rich waste water. Smith *et al*. (1991) suggest that it might have been a minor sink for nitrate in a sand and gravel aquifer contaminated with a plume of treated sewage effluent. A narrow plume of nitrate was identified at the top of the effluent plume, where ammonium from the source had oxidised. Ammonium taken from within the nitrate plume had significantly enriched $\delta^{15}N$ isotope composition (Section 4.7.1) compared to ammonium from the source or in the main plume. Nitrate was also significantly enriched in $\delta^{15}N$, suggesting that the nitrate may have been the source of the ammonium.

Kelso *et al.* (1999) used organic carbon treatments in an attempt to stimulate DNRA in river sediments under anaerobic conditions. It was found that glycine (an amino acid) and glucose stimulated some formation of ammonium, though for glycine this may have been partly through mineralisation of the amino group, while most nitrate was denitrified or converted to biomass. Acetate and formate, both fermentation products of organic wastes, only reduced nitrate concentration by denitrification or conversion to biomass.

Although elevated nitrite levels do not tend to occur by denitrification, they are common if nitrate reduction is preceded by DNRA. In particular, DNRA occurs when high concentrations of nitrate inhibit the nitrite reductase enzyme (Kelso *et al.,* 1997). High levels of nitrite may therefore be indicative that DNRA is the dominant nitrate reduction process in a system. DNRA should not be thought of as a sink for nitrate nitrogen, but perhaps as a process to be aware of because it can lead to high concentrations of groundwater or surface water nitrite.

4.9.2 Assimilation of nitrate into microbial biomass

Although many heterotrophic micro-organisms can assimilate nitrate for growth, it appears that in the presence of ammonium, the latter compound is taken up preferentially for growth (Hill, 1996). However, in some environments, there is evidence that biomass can become an important mechanism for nitrogen uptake. For example, Kelso *et al*. (1999) showed that in the presence of some organic substrates, up to 50 per cent of nitrogen depleted from groundwater could be converted to biomass.

Except in systems where microbial biomass development is extensive (for example, following a release of pollutant organics into the environment or during active bioremediation (Hu *et al*., 2000)), it is difficult to foresee many cases where microbes will assimilate a significant amount of nitrate. For example, a kilogram (dry weight) of hydrocarbon-contaminated aquifer may contain 2.5×10^{10} cells (Holm *et al*., 1992). Assuming that the dry weight of a ‘typical’ bacterial cell is one picogram, this corresponds to a total biomass of 0.025 g/kg. If the porosity of the sediment is 30 per cent, and its bulk density 1600 kg/m^3, data in Table 4.4 shows that this corresponds to a microbial N load of only 4 mg/l as N.

5 Attenuation of nitrate in hydrogeological environments

Sections 3 and 4 describe the processes involved in nitrate attenuation. The following section gives examples of observed attenuation in the field.

5.1 Unsaturated zones

5.1.1 Physical processes

Solute retardation mechanisms in the unsaturated zone were discussed in Section 3.1. In a homogeneous porous medium (of primary porosity only), the solute is dispersed via a tortuous path through partially saturated pores. In a fractured porous medium the transport process is more complex, with the fractures acting as flow conduits when they are sufficiently saturated, and pores able to act as stores and secondary sources of solute. Chemical concentration gradients may also cause diffusional exchange of solute between fracture water and matrix pore water. These processes act irrespective of the solute – that is, they are not specific to nitrate.

UK studies of nitrate movement through the unsaturated zones of aquifers have tended to concentrate on the Chalk because of its importance as the UK's principal aquifer. These studies are summarised in Table 5.1, along with rates of nitrate solute movement (although it is recognised that some rates may be influenced by attenuation as well as physical processes). At a site in Hampshire, Wellings (1984) showed by physical measurements that fracture flow was not observed in the uppermost 3 m of the unsaturated zone of chalk, and estimated that nitrate moved with the advective downward flow at a rate of 850 mm/a. Barraclough *et al*. (1994) studied a site in Berkshire and found that, at least in the upper 6 m of chalk, fracture flow was also unimportant; however, the 1960's tritium pulse was not detected at the depth predicted by matrix flow, suggesting that fissure flow might have occurred deeper than 6 m.

Barraclough *et al*. (1994) nevertheless found that the rate of solute movement (833 mm/a) was greater than that which would be predicted if the recharge rate were divided by the saturated porosity (440 mm/a). It was suggested that transport only occurs in half the pores, principally the largest pores with diameters greater than 0.7 to 0.8 μm. In contrast, Foster and Bath (1983) found that water beneath 1.5 m in the unsaturated zone of Chalk near Cambridge was essentially immobile. Beneath this, solute movement occurs only when lateral molecular diffusion brings immobile pore water into micro-fissures. Solute is flushed from the micro-fissures when they are saturated, the rate and frequency being dependent on variations in duration and intensity of rainfall. Similarly, at test sites on Cambridgeshire Chalk, Carey and Lloyd (1985) found that observed nitrate peak movement was best modelled primarily by advective flow through the matrix, but with a component of bypass flow (10 per cent) that caused more rapid breakthrough of the solute front.

Table 5.1. Rate of nitrate pulse movement in the unsaturated zone of UK Chalk

Location	Rate of solute movement (mm/a)*	Reference
Hampshire (Bridgets Farm)	850 (*r*)	Wellings, 1984
East Yorkshire	833 (*p*)	Foster, 2000
Berkshire	833 (*p*)	Barraclough *et al*., 1994
Cambridgeshire (Fleam Dyke)	~500 (*p*) over 3 years	Foster and Bath, 1983
Cambridgeshire (Fleam Dyke)	455 (*p*) over 11 years	BGS, 1991
Cambridgeshire	450 (*r*)	Carey and Lloyd, 1985
Cambridgeshire	200 (*p*)	Andrews *et al*., 1997b

* (*r*) = based on recharge rate and effective porosity, (*p*) = measured rate of nitrate peak movement

All the examples above illustrate cases in which ambient recharge rates were applied – in other words, the recharge derived from natural rainfall only. Soakaways and irrigation develop a hydraulic surcharge that drives solutes through the unsaturated zone. Edworthy *et al*. (1978) list a number of sites on the chalk of South East England at which soakaways have been operating, and show that the typical rate of downward solute movement in these cases is up to approximately 1000 mm/a.

Significant dispersion of solute fronts and peaks, perhaps indicating a greater contribution from fissure flow, has been observed in a number of pore water profiles from the unsaturated zone of chalk (Barraclough *et al*., 1994; BGS, 1991), although it is not always observed over the timescale of investigation (Wellings, 1984). The effect of dispersion in the long term will be to reduce the concentrations of nitrate reaching groundwater, while prolonging the impact.

5.1.2 Unsaturated zone denitrification: shallow superficial aquifers

Air within the pores of soil and the unsaturated zone generally provides a more readily replenished supply of oxygen than the oxygen dissolved in groundwater (where the oxygen concentration is limited to approximately 10 mg/l, depending on the temperature). Superficial aquifers such as sands and gravels are readily replenished because of their typical shallow settings. Denitrification in the unsaturated zone of many aquifers is therefore not commonly observed, except where exchange of air is limited by low permeability lithologies or where there are very high concentrations of electron donors such as organic wastes. 'Aerobic' denitrification should, however, generally be recognised in unsaturated zones, with the existence of anaerobic micro-sites within generally aerobic environments (Lloyd *et al*., 1987). Detecting denitrification in the unsaturated zone is not trivial. Principal field methods include: long-term monitoring of the fate of nitrate and associated mass balances; establishing products of denitrification (N_2, N_2O); monitoring stable isotopes ($^{15}N/^{14}N$) in N_2 or nitrate; and use of the 'acetylene block' technique (BGS, 1999).

In the unsaturated zone denitrification mostly occurs in or near the soil zone, principally because it is the region with the highest concentrations of organic carbon (Brady and Weil, 2002). The process arises primarily from the decay of dead vegetation, though plant exudates may also contribute. In the UK inputs of DOC vary seasonally, with much greater fluxes in autumn from plant die-off. Warm, wet, organic-rich, autumn soils also provide an environment for rapid denitrification (Addiscott, 1996). The decreasing concentrations of DOC down through the unsaturated zone arise from their gradual mineralisation to carbon dioxide.

Cannavo *et al*. (2004) studied the 1.6 m thick unsaturated zone of a calcareous organic silty clay to assess the amount of denitrification beneath a field of decomposing crop residues. The DOC concentration of the pore waters was found to decrease downwards, along with the denitrification activity (Figure 5.1). As a consequence, the mean concentration of nitrate leached from the base of the unsaturated zone was approximately 14 mg N/l, despite a mean input concentration of approximately 240 mg N/l. The denitrification rate appeared to be limited in the top 90 cm by the amount of carbon available, but beneath this it was limited by the amount of nitrate draining from the upper soil horizons.

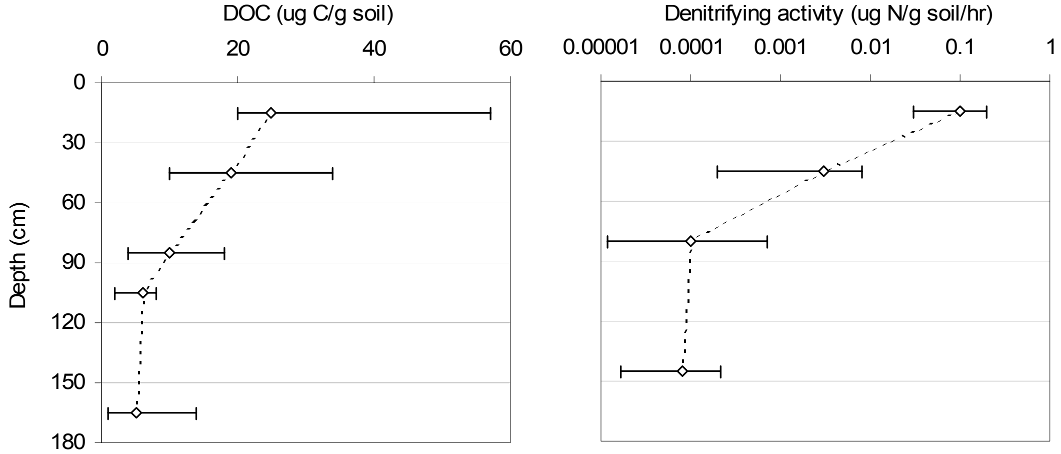


Figure 5.1. Variation in DOC and denitrification activity with depth (after Cannavo *et al*., 2004).
Values on these charts were obtained by eye from charts in Cannavo et al. (2004) and are approximate.

In typically aerobic unsaturated zones, denitrification may occur within anaerobic microenvironments formed in the pore spaces within clumps of finer-grained particles (aggregates) or in fine-grained sediments. Solutes diffuse in and out via the interaggregate spaces, or in lower permeability environments, interconnected macropores. Casey *et al*. (2004) found that denitrification in the soil adjacent to macropores was one to two orders of magnitude greater than in the bulk sediment in organic-rich riparian sediments. The frequency and size of macropores tends to decrease with depth, which reduces the ability for reactants to be transported to anaerobic microenvironments. Transport of reactants to the microbial fauna was identified as the rate limiting factor by Jörgensen *et al*. (2004), who observed that denitrification rates decreased with increasing depth in the subsoil. First order half-lives from the surface to 3.5 m depth increased from seven to 35 days beneath forest soil and from one to seven hours beneath agricultural soil; the difference in rate between the two sites was ascribed to the difference in concentrations of water soluble carbon and denitrifying bacteria.

5.1.3 Unsaturated zone denitrification: major UK aquifers

Several UK studies have assessed the transport and attenuation of nitrate through the unsaturated zone of major UK aquifers via cored profiles (see Table 5.1 for chalk). This research has principally been undertaken by the British Geological Survey (BGS) and to a lesser extent the Water Research Centre (WRC). Parker *et al*. (1991) and BGS (1999) provide reference to much of this research. This wealth of unsaturated zone core pore water data has been drawn together by BGS (1999) into a database of pore water nitrate (and chloride and sulphate) profiles for the period 1976-98 from 104 boreholes drilled at 14 sites.

5.1.4 Unsaturated zone denitrification – Cretaceous Chalk

Historical nitrates research has been carried out at a number of sites; for example, the Bridget's Farm site near Winchester on Hampshire Chalk has been studied since the 1970s (BGS, 1999; Wellings, 1984; Whitelaw and Rees, 1980; Young and Gray, 1978; Young *et al*., 1976). Much research has also been conducted in East Anglia. Data from the Hampshire and East Anglian Chalk, as well as a 1990s research site established on Wiltshire Lower Chalk (Gale *et al*., 1994), are discussed below.

BGS (1999) use part of the core pore water profile database (referred to in Section 5.1.3) to investigate denitrification via a mass balance approach. The most extensive set of repeated BGS drillings (BGS, 1991) carried out at five chalk sites in East Anglia was used and the successive decrease in nitrate content of a given 'packet' of water tracked downward through the unsaturated zone over time. The results, however, proved inconclusive as the 1990 repeat drilling tended to show a rise in nitrogen in the unsaturated zone that may relate to spatial variability between nearby profiles as well as complex flow mechanisms not considered. Although the approach was of limited value in estimating small amounts of denitrification, it nevertheless provided evidence that denitrification is probably not of major significance in the chalk unsaturated zone (BGS, 1999).

Microbiologically-based supporting studies did, however, suggest there is microbial transformation of nitrogen species in the unsaturated zone additional to that found in the overlying soil zone. As a typical bacterium size is 1 to 5 μm and chalk pore sizes are 0.1-1.0 μm, most bacterial activity in chalk will be restricted to the fissure walls and not occur within the chalk pore matrix (Foster *et al*., 1995; Whitelaw and Edwards, 1980; Johnson *et al*., 1998). Chalk-based studies also indicated relationships between carbohydrates and other chemical parameters and suggested that microbial transformations of nitrate, nitrite and ammonium may be occurring (Whitelaw and Edwards, 1980). Supporting studies to identify bacteria in the unsaturated zone were then undertaken (Whitelaw and Rees, 1980) on the Middle Chalk at Deep Dean, Eastbourne (a permanent grassland site) and the Upper Chalk at Bridget's Farm, Hampshire (a fertilized arable site). These indicated that:

- both nitrate-reducing and ammonia-oxidising bacteria are present throughout the Deep Dean chalk;
- there was an approximate inverse relationship between these bacteria populations at specific horizons;
- a peak in nitrate-reducing populations also coincided with peaks in nitrate and nitrite concentration.

This, together with a correlation between carbohydrate solids in chalk and nitrite in chalk pore water at Deep Dean (Whitelaw and Edwards, 1980), provides a strong indication of denitrification. However, actual activity was not proven or denitrification rates estimated.

In contrast, microbiological data (Whitelaw and Edwards, 1980) from the Bridget's Farm site showed only elevated populations of nitrate-reducing bacteria at 30-40 m depths coincident with low concentrations of pore water nitrate. Much of the unsaturated zone was occupied by ammonium-oxidising bacteria, a lack of nitrate-reducing bacteria and aerobic conditions in which little, if any, attenuation by denitrification was observed, or indeed expected. A more recent study at Bridget's Farm (BGS, 1999) focused on the existence of gases. Evidence from carbon isotopes suggested most of the unsaturated zone carbon dioxide actually originated from bacterial breakdown of organic matter in the overlying soil. Although some nitrous oxide was detected and might suggest denitrification, the source of nitrous oxide could have been the soil zone or the nitrification of ammonia. Marginally elevated N_2/argon ratios indicated an excess N_2 of around 0.5 per cent, indicative of a low rate of denitrification of only 0.4 mg N/l, compared with the mean unsaturated zone concentration of 26 mg N/l (a 1.5 per cent loss in nitrate). It was not fully clear if this denitrification occurred in the soil or unsaturated zone. Slightly negative values of $\delta^{15}N\text{-}N_2$ supported the above, inferring minor denitrification at rates that were not quantifiable. Examination of the $\delta^{15}N$ composition of infiltrating nitrate relative to the underlying groundwater nitrate similarly confirmed denitrification was not a significant process in the unsaturated zone.

The early 1990s study by Gale *et al*. (1994) examined evidence for denitrification in core profiles at the Ogbourne St George, Wiltshire chalk site. The site exhibited a nitrate front moving downward at ~0.8 m/a, with any input nitrate peaks smoothed by the wide range in seasonal water table fluctuation (~ 5-24 m below ground surface). Nitrate, dissolved oxygen and organic carbon, however, were all thought to be replenished annually by a component of rapid fissure flow to the water table. Similar denitrification findings to the above chalk sites were measured at Ogbourne; for instance, nitrite, N_2O and excess N_2 were measured in low amounts and estimated to represent a few percent decrease in the annual nitrate load. Supporting microbiological studies indicated denitrifiers were present at all depths, with greatest numbers (7×10^5 CFU/g) at 7 m, just below the water table maximum. Lab microcosms on core material indicated a 2 per cent nitrate conversion over 21 days, a rate comparable to field estimates of denitrification, though organic carbon supply was limited in the closed batch system used.

The above findings may be expected to have reasonable generic applicability to chalk unsaturated zones elsewhere. Indeed, none of the studies on agricultural nitrate described in Table 5.1 positively identified denitrification as a significant attenuation mechanism except in the soil zone. At best, low rates of denitrification may generally be expected, given that the unsaturated zone of chalk has relatively good contact with the atmosphere via its fracture network and has a low level of in situ electron donors.

Although low oxygen conditions may be present in the chalk matrix, the fine pore matrix will exclude many bacteria, causing bacterial activity to be largely restricted to fracture sites where it is difficult to establish anaerobic conditions necessary for denitrification; the exception would be if there were substantial labile carbon inputs. For example, Gooddy *et al*. (2002) showed that denitrification was occurring in the unsaturated zone of chalk beneath unlined cattle slurry lagoons, because of the substantial entry of organic carbon. Edworthy *et al*. (1978) have similarly observed denitrification in the unsaturated zone of chalk beneath an effluent lagoon.

5.1.5 Unsaturated zone denitrification – Permo-Triassic Sandstone

The most significant nitrate attenuation study of the Permo-Triassic Sherwood Sandstone unsaturated zone has been undertaken by BGS at the ADAS Gleadthorpe Land Research Centre (BGS, 1999). Gleadthorpe was one of the sites included in the 1970s nationwide nitrates survey (Young and Gray, 1978) and is located on the Permo-Triassic Sherwood Sandstones, near Mansfield in Nottinghamshire. These have an unsaturated zone thickness of 8-12 m, the rather large water table variation being due to pumping from a number of nearby public water supply abstraction boreholes. Similar methods were employed at this site to the Bridget’s Farm site. In short, denitrification was not identified as a significant process, with only limited evidence for the process obtained, listed as follows (BGS, 1999):

- some evidence of nitrate depletion just beneath the water table in one borehole, where nitrate concentrations declined with depth while nitrite increased;
- in one near-surface sample, $\delta^{15}N$-N_2 and N_2/Ar data were consistent with minor denitrification;
- denitrifying bacteria were found at all depths, indicating the potential for denitrification;
- N_2O was above atmospheric concentrations, though it was unclear whether this was due to soil-zone denitrification (known to be very active) or nitrification from ammonium - sufficient (small) quantities of the latter were present throughout.

The N_2/Ar data suggested a maximum amount of denitrification of 0.8 mg N/l, compared with an average unsaturated zone concentration of 37 mg N/l (a loss of two per cent).

For Sherwood Sandstone unsaturated zones, BGS (1999) concluded from their Gleadthorpe study that the following would militate against denitrification being significant:

- low supplies of labile organic carbon, leading to low rates of microbial activity and little chance of the development of anaerobic hot spots;
- although pore sizes in sandstones were tens to hundreds of microns, allowing (in contrast to the chalk) bacteria in the porous matrix, the pores were less water-saturated (that is, better drained), allowing the entry of oxygen – thus, the establishment of conditions conducive to anaerobic denitrification was less likely.

A further core profile study on Sherwood Sandstone at the Boughton site, Nottinghamshire, revealed similar findings (Gale *et al*., 1994). Minor denitrification activity was suggested by: depth-variable nitrite concentrations that implied low conversions of between 0.3 and 1.5 per cent of the nitrate present; an absence of excess N_2; and only occasional sporadic detection of N_2O. Product and microbiological data suggested greatest denitrification occurred just above the 24 m deep water table. Mean dissolved oxygen concentrations were high at 8.5 mg/l, supporting the hypothesis of the presence of anaerobic microenvironments.

5.2 Major UK aquifers – saturated zone denitrification

Overviews of saturated zone nitrate transport and attenuation are provided, for example, by Korom (1992), Foster (1986), Hiscock *et al*. (1991) and Parker *et al*. (1991). Investigation of the occurrence of nitrate attenuation in the saturated zone of UK aquifers has, in general, received less attention than the unsaturated zone. Long unsaturated zone migration times on the order of decades and the ease of study of vertical migration via a single core in the unsaturated zone have contributed to focus on the latter.

For unconfined major aquifers, most nitrate investigations have focused on spatial delineation of nitrates, in particular the vertical stratification in groundwater (Parker *et al*., 1991). Nitrate penetration depths into the saturated zone can be significant, particularly where unsaturated zones are thin and have been influenced by large public supply abstractions. For example, Sherwood sandstone at Carlton in South Yorkshire shows nitrate to depths around than 100 m within a major well field (BGS, 1985; Parker *et al*., 1991) and through a significant thickness of Norfolk Chalk (Parker *et al.,* 1991). Proactive seeking of evidence for denitrification in unconfined zones of saturated aquifers, however, appears limited. This is not too surprising, given that the widespread aerobic conditions do not favour denitrification.

Studies on major aquifers with considerable dissolved organic carbon do demonstrate the occurrence of unconfined zone denitrification. The River Glen which runs across the Lincolnshire limestone near Bourne, Lincolnshire, recharges the aquifer year-round because of the proximity of large public water supplies (Rushton *et al*., 1982). In winter, nitrate-rich surface waters recharge the aquifer, leading to the development of nitrate plumes emanating from rivers and swallow holes, while in summer the rivers recharge DOC-rich effluent from small sewage treatment works. Roberts and McArthur (1998) show that the anaerobic conditions created by the carbon inputs promote denitrification; the DOC contributes some small amount of denitrification, though reduction of in situ sulphide contributes much more.

Confining layers of impermeable material limit the entry of oxygenated recharge water and the diffusion of atmospheric oxygen to aquifers. A supply of nitrate and a suitable electron donor provide conditions more favourable to denitrification. Confinement may be regionally extensive via thick aquitards or more sporadic, occurring locally below discontinuous low permeability drift or till deposits. The following sections focus on denitrification under such confined, low oxygen conditions; other nitrate attenuation mechanisms have not been described in the field-based literature. Most UK studies have traced the transition from high nitrate in unconfined conditions to nitrate-free conditions within a confined system, for example Norfolk Chalk (Parker and James, 1985), South Yorkshire Sherwood Sandstone (Parker *et al*., 1985), and Lincolnshire Limestone (Lawrence and Foster, 1985).

Although there is a considerable amount of data on nitrate in UK major aquifers and widespread evidence of low nitrate below confined layers suggestive of denitrification, studies that have specifically looked for evidence of denitrification and detailed transect or vertical profile studies are rare. The next sections focus on the available studies within aquifer units.

5.2.1 Cretaceous Chalk

Chalk in the UK contains more than 99 per cent calcium carbonate and the matrix content of electron donors (organic carbon, Fe^{2+}, Mn^{4+}) is very low (Gooddy *et al*., 2002). Consequently, redox reactions such as denitrification are expected to be low. With a very small pore throat size of approximately 1 μm, chalk will only maintain microbial populations on fracture surfaces (Tompkins *et al*., 2001). The dual porosity nature of the formation also makes solute transport relatively slow (Section 3.2), with reaction rates likely to be determined by slow diffusion of solutes into the fissure spaces from the matrix.

Several field studies of UK chalk have failed to identify any significant denitrification, at least over the time since nitrate inputs increased in the mid-20th Century (Edmunds *et al.*, 2001; Howard, 1985), suggesting that chalk denitrification is minimal. Similarly, calibrated nitrate transport modelling studies have not found it necessary to cover nitrate attenuation (Carey and Lloyd, 1985).

The most typical feature of many regional aquifer nitrate assessments is the gradual lowering of nitrate concentration in groundwaters in the direction of flow from unconfined recharge zones to increasingly confined zones down dip. For example, the East Yorkshire Chalk aquifer shows elevated nitrate concentrations over much of the agricultural unconfined area, with nitrate decreasing to undetectable concentrations over distances of less than approximately 5 km beneath the confining layer (BGS, 1996). A little further south, Howard (1985) presents similar data for the North Lincolnshire Chalk in which nitrate concentrations drop from 10-25 mg N/l to less than 2 mg N/l over 9 km, while the dissolved oxygen content drops from 10 mg/l to 1 mg/l over a similar distance.

As Howard (1985) states, it is tempting to simply invoke a conceptual model implying rapid denitrification in the direction of groundwater flow, particularly as the redox potential lowers in the direction of flow, the presence of nitrate-reducing bacteria was confirmed in all sampled sites and thermodynamically, nitrogen gas is more stable than nitrate in moderately oxidising waters. However, more thorough assessment of supporting data is required to prove denitrification occurs. Howard (1985) concluded, via complementary assessment of hydrochemical water types and isotope data that the lowering of nitrate in the direction of flow was caused by the gradual mixing of water from different origins, in other words dilution with older, oxygen-deficient, low nitrate groundwaters rather than significant denitrification. However, possible evidence of denitrification was found in water over 4000 years old via the presence of reduced nitrogen species. Howard’s main conclusion was that denitrification cannot be relied upon to reduce elevated nitrate concentrations in modern recharge waters.

Denitrification has nevertheless been observed in some chalk areas. For example, Hiscock *et al.* (1989) identified a discrete layer (approximately 50 cm thick) of flinty sand within Norfolk Chalk, which was found to be chemically reducing and to contain high numbers of denitrifying bacteria. Foster *et al*. (1985) also identified denitrifying bacteria at the top of the Norfolk Chalk (Mattishall) at a location where it is overlain by sands and tills. The effects of denitrification were observed in the chalk groundwater, but whether this was caused by reaction in the superficial deposits or chalk, or both, was not determined.

Tompkins *et al*. (2001) explain that the main parameters controlling *in situ* bioremediation of nitrates in UK chalk are as follows:

- transport of bioavailable carbon substrate and nitrate within the fissure system;
- rate of diffusion of nitrate from the matrix to the fissures;
- fissure density and fissure properties;
- rate of microbial growth and activity.

Once dissolved oxygen is depleted, flow processes determine whether significant denitrification can occur. Tompkins *et al*. (2001) propose that *in situ* bioremediation of nitrates is viable given amenable conditions. However, given that the chalk is a very heterogeneous formation, it is likely that such conditions are not in evidence everywhere.

5.2.2 Jurassic Lincolnshire Limestone

Limestone formations of various ages, such as Carboniferous and Jurassic, occur across the UK. All have a dual porosity nature like Cretaceous Chalk, but generally have lower matrix porosities and greater fracturing and in the extreme are karstic. Unlike the Chalk, Lincolnshire Limestone does contain appreciable quantities of organic carbon and, in more argillaceous units, pyrite (Bottrell *et al.,* 2000). The Jurassic Lincolnshire limestone has been extensively used for supply and is at risk from nitrates from extensive agricultural land use. Major abstractions in the confined (as well as unconfined) aquifer since the 1950s have had the potential to draw nitrate contamination down dip from impacted unconfined areas. Hydrochemical changes across the unconfined-confined transect were identified by several studies in the 1980s (Edmunds and Walton, 1983; Lawrence and Foster, 1986; Bishop and Lloyd, 1990; Wilson *et al*., 1990). Figure 5.2 presents a schematic cross section from Lawrence and Foster (1986).

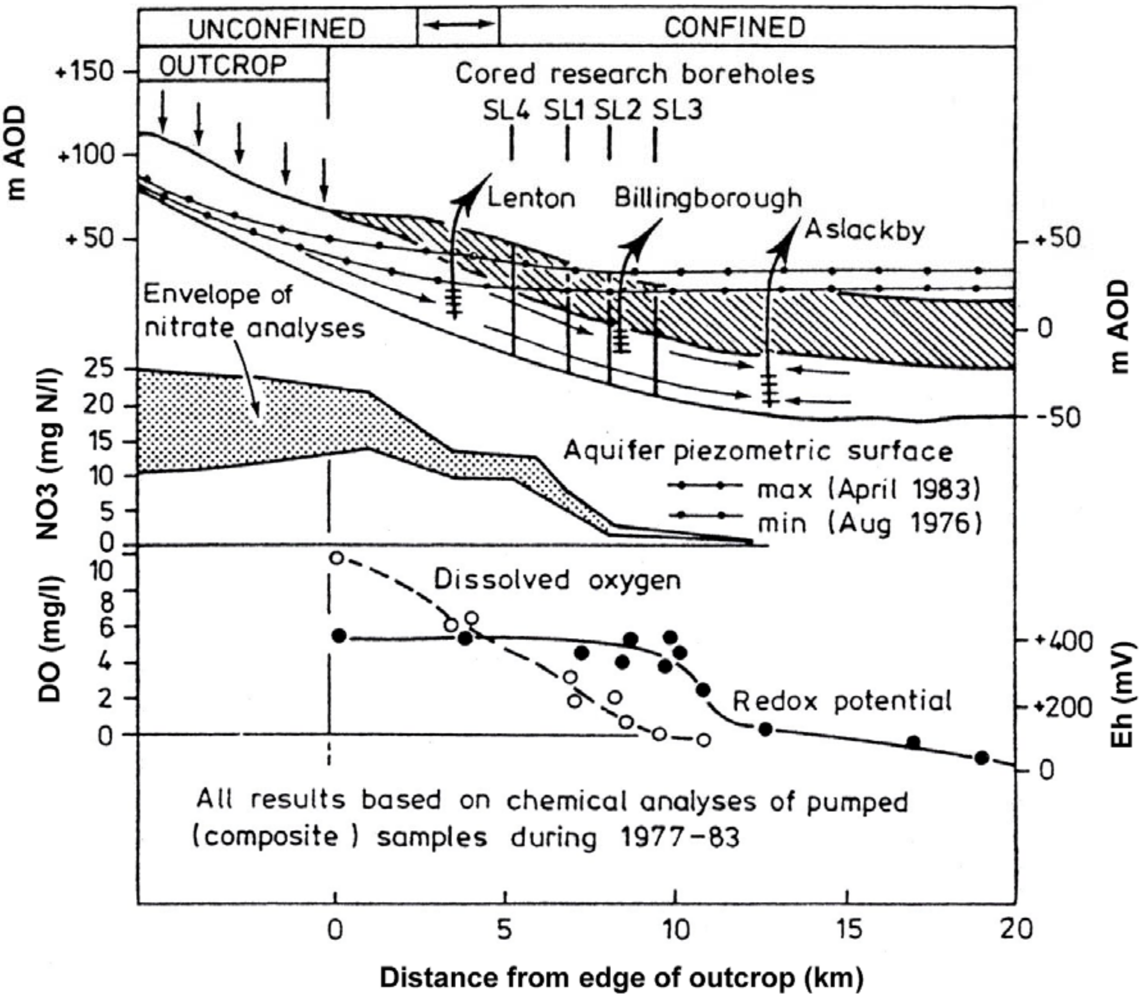


Figure 5.2. Changes in dissolved oxygen, nitrate and redox potential in the Lincolnshire Limestone aquifer (after Lawrence and Foster, 1986).

As shown in Figure 5.2, the limestone aquifer exhibits a classic down-dip redox transition in the shallow confined zone of the aquifer, as dissolved oxygen, then nitrate and sulphate, are reduced. Such trends are generally ascribed to biologically mediated reactions with aquifer organic matter and are cited as evidence for denitrification. However, as discussed above (Howard, 1985), mixing of waters can potentially cause such trends, and confirmatory evidence for denitrification should be sought. Evidence presented below counteracts the mixing argument.

Foster *et al*. (1985) list nitrate, tritium and bacterial activity data for cores taken from the geological cross section presented in Lawrence and Foster (1986) reproduced as Figure 5.2 that supports denitrification. For example, thermonuclear tritium had penetrated further down dip from outcrop than both nitrate and dissolved oxygen. Wilson *et al*. (1990) also present evidence for denitrification that includes concentrations of excess nitrogen and isotopic ratios. Lawrence and Foster (1986) provide evidence of denitrification occurring within the matrix immediately adjacent to fissures, possibly because the matrix was more open in the weathered zone adjacent to the fractures. No denitrifying activity was observed in matrix pore water away from the fissures. The control exerted on bacterial reduction reactions by the dual porosity characteristics of

Lincolnshire limestone was later explored by Bottrell *et al.* (2000). By analogy with sulphate-reducing reactions in the aquifer, it was concluded that the potential for denitrification in the shallow confined zone is poor. Reaction rates will be limited by lack of an electron donor (organic carbon and/or sulphide) in the fissures; were nitrate to diffuse into the pores where organic carbon and sulphides are present, nitrate-reducing bacteria would be excluded by the narrow pore throats of the matrix (similar to chalk).

This is not to say that all dual porosity aquifers are unlikely to support denitrification. Seiler and Vomberg (2005), for instance, found that in a karstic reef limestone in the Jura of Southern Germany, the pore size was sufficient (~50 μm) for biofilms to form within them. In fact, high flow velocities in the fractures tend to inhibit growth of the biofilms by shear stresses.

5.2.3 Permo-Triassic Sherwood Sandstone

Despite being relatively deficient in organic carbon (Table 4.2), the Permo-Triassic Sandstone aquifers of the UK can exhibit denitrification where they are confined and flow is sufficiently slow for long contact time between the aquifer and water. These hydrogeological conditions may occur where the aquifer is confined by the Mercia Mudstone Group, or by thick deposits of low permeability glacial till.

The South Yorkshire Sherwood Sandstone aquifer system is characterised by variable drift coverage of the sandstone, with many of the major water abstractions located on sandstone 'islands' that protrude through the drift (BGS, 1985). The aforementioned deep (50–100 m) penetration of nitrate in these unconfined portions contrasts significantly with immediately adjacent areas confined beneath low permeability superficial deposits that contain near undetectable concentrations of nitrate throughout the water column (BGS, 1985; Parker *et al*., 1991). The confined water appears to be old, and therefore denitrification needs to have occurred only very slowly, assuming that some nitrate was present in the original recharge waters. It is significant that an abrupt change in nitrate quality has been maintained at the confined–unconfined boundary, despite the presence of a major abstraction in the confined zone. It would appear that denitrification has not been proven at the site, although Parker *et al*. (1991) refer to an unpublished report considering the prospects for denitrification in this case.

Wilson *et al.* (1990) explore the potential for denitrification of groundwater in the unconfined East Midlands Sherwood sandstone aquifer. This aquifer has less drift cover than the South Yorkshire aquifer mentioned above and therefore waters are more oxygenated. Consequently, widespread denitrification is not observed in the aquifer except in two isolated locations. These coincide with relatively low oxygen concentrations (6.2 mg/l and 3.0 mg/l), but no comment on these special hydrogeological conditions is made in the paper.

Cartmell (1997) demonstrated denitrification in Permo-Triassic sandstone material when 10 mg C/l organic carbon was added to flow-through microcosms. With a source of dissolved organic carbon from sewer leakage, pollutant nitrates in the Triassic Sherwood Sandstone beneath Nottingham are readily denitrified (Fukada *et al*., 2004). Similarly, phenol and *p*-cresol are readily degraded by denitrification of agricultural nitrate pollution in a contaminant plume in the West Midlands (Spence *et al*., 2001).

5.3 Shallow, permeable aquifers

Many studies demonstrate that denitrification can occur in shallow, permeable aquifers despite these being superficial and predominantly aerobic (see references below). The main obstacle to denitrification would be expected to be the establishment of anaerobic conditions, but with suitable geological conditions or excess concentrations of electron donors it has nevertheless been observed. In many systems, denitrification does appear to be limited by the availability of electron donors, most often organic carbon, even in wastewater-derived plumes (Section 4.4.1). Many of the shallow aquifer studies have been conducted in North America with, for example, Gillham and Cherry (1978) being amongst the earliest researchers to provide good evidence of denitrification in groundwater.

In a US study, Spalding *et al*. (1993) illustrate some of the effects of geological heterogeneity on denitrification. A large plume from a sludge injection field in a generally sandy aquifer is vertically split by a lens of clay into deep and shallow sections. There is leakage of nitrate through the thin clay lens but the deep plume quickly becomes anaerobic and denitrification occurs, while the shallow plume stays aerobic and no nitrate is lost (Figure 5.3). The maximum vertical extent of the plume is shown by the chloride profile, which reaches 12 m below the base of the lens; meanwhile, elevated nitrate only reaches to 6 m beneath the lens. The heavier $\delta^{15}N$ signature of the groundwater within the plume, but beneath the zone of high nitrate, demonstrates clearly that denitrification has occurred within this region.

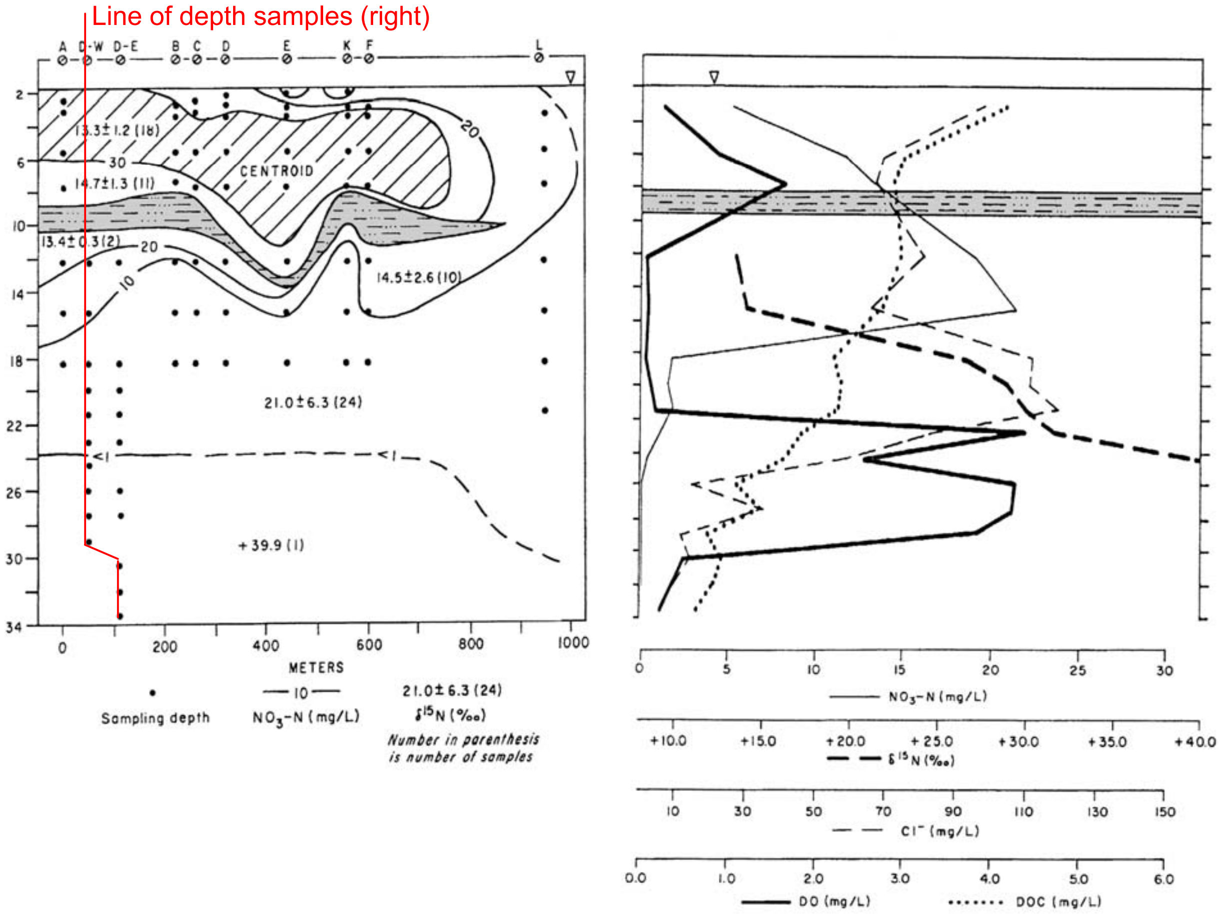


Figure 5.3. Vertical sections of nitrate, δ^{15}N, chloride, dissolved oxygen and DOC in a septic plume in a heterogeneous aquifer (after Spalding *et al.*, 1993). Shaded geology represents a clay lens.

Geological control over denitrification is also demonstrated in two Maryland catchments studied by Böhlke and Drever (1995). The geological succession of the study area includes a fluvial sand and gravel unit (the Pensauken Formation) overlying a marine sand (the Aquia Formation), with a glauconitic clayey sand unit at its base. The two catchments have similar geologies and land uses, but widely differing surface water nitrate concentrations: low (2-3 mg N/l) in the Morgan Creek catchment and higher (9-10 mg N/l) in the Chesterville Branch catchment. The difference in δ^{15}N values between the two surface waters is four to five in the Chesterville Branch, while seven to ten measured in Morgan Creek shows that denitrification has occurred in the groundwaters discharging to Morgan Creek. The key difference is due to the dip of the strata here: as illustrated in Figure 5.4, much of the baseflow to the Morgan Creek catchment has to flow through the clayey glauconitic unit at the base of the Aquia Formation, whereas little of the baseflow to the Chesterville Branch catchment does. It appears that the clayey Fe^{2+}-rich sediment hosts denitrification reactions; however it is not discussed whether reduction is driven by organic carbon or reduced iron.

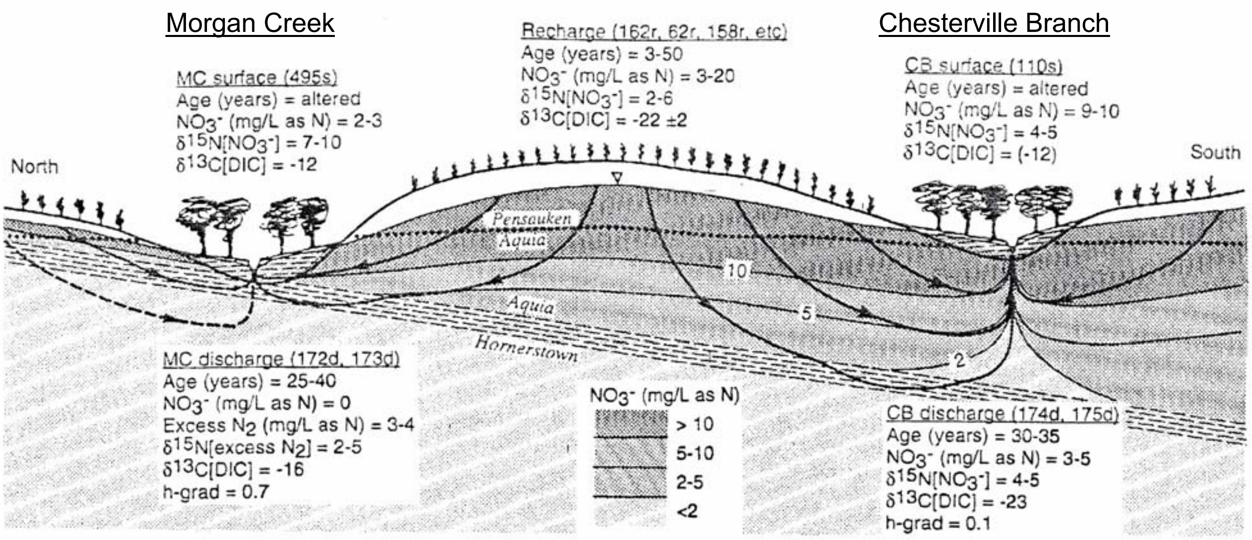


Figure 5.4. Schematic cross section through two shallow catchments in Maryland (after Böhlke and Drever (1995).

Depth stratification of nitrate concentrations is often observed in shallow aquifers, with both instances of low nitrate waters being uppermost in the water column, and the opposite, being observed. Stratification where low nitrate concentrations occur above high concentrations is most commonly influenced by dissolved organic carbon from the unsaturated zone above. Where an influx of DOC in recharge reaches the water table, and if it is sufficient to deplete concentrations of dissolved oxygen, it can cause denitrification at the top of the water column (for example, Starr and Gillham, 1993). A key implication of this is that, for denitrification to occur, sufficient DOC must reach the water table and not be mineralised in the unsaturated zone. Pabich *et al*. (2001) found that for a sand and gravel aquifer, with unsaturated zone thickness of less than 1.25 m, DOC concentrations exceeded 20 mg/l, while with unsaturated zone thickness of greater than 5 m, DOC concentrations never exceeded 2 mg/l. Further attenuation occurred beneath the water table and DOC concentrations declined exponentially, with high nitrate concentrations correlating with low DOC, implicating carbon-limited control over denitrification in the groundwater.

A number of scenarios may cause the uppermost levels of groundwater to be high in nitrate, with depleted concentrations beneath. The most simple scenario is that deeper waters flow along a longer flow path and pre-date applications of artificial fertiliser or are sourced from an area without applications. Alternatively, there may be a redox profile down through the aquifer, since dissolved oxygen must be depleted from infiltration before denitrification can commence (for instance, Puckett and Cowdery, 2002). In cases where the concentration of the electron donor may be limiting, nitrate may have been depleted in the upper levels of the aquifer, leading to denitrification occurring only in the lower parts of the aquifer, where there is a slower throughput of groundwater (Böhlke *et al*., 2002; Böhlke and Drever, 1995). In a survey of farm wells in the sand and gravel aquifers of Ontario, Goss *et al*. (1998) show that, on average, deeper farm wells yield water with lower nitrate concentrations. The reason was not discussed in detail, but is thought to be due to the interception of longer flow paths, along which groundwater has been subject to a greater duration of denitrifying activity.

Other plumes, especially those from septic systems, tend to ‘dive’ into the aquifer because of the relatively fresh water recharging at the water table (Robertson *et al*., 1991, Harman *et al*., 1996; MacQuarrie *et al*., 2001a). This may sometimes combine with the geological heterogeneity to form a complex plume shape: for example, Kelly (1997) describes a diving plume that reaches a nitrate-reducing zone lower in the aquifer.

None of the above studies were conducted in the UK. It would appear that denitrification studies in shallow UK aquifers have been limited to studying the breakdown of hydrocarbon contamination, possibly because of the lack of widespread water supply shallow aquifers (the Thames Valley Gravels forming the most prominent supply) and/or the lack of significantly sized septic waste discharges (compared to North America for example) that permit the development of shallow anaerobic conditions.

5.4 Aquitards and glacial tills

Study of nitrate attenuation in aquitards is important because in many areas of the UK, important public water supply aquifers are overlain by low permeability, often clay-rich strata (such as glacial till) which separate the aquifer from near-surface nitrate contamination. The depositional environments of aquitard sediments are distinctly different from those of the aquifers, so the type and quantity of electron donors may be different. Typically, the concentration of electron donors in aquitards is far in excess of adjacent aquifers and can provide significant protection to underlying aquifers if the physical conditions bring the reactants and micro-organisms in contact (Robertson *et al*., 1996; McMahon, 2001; Rodvang and Simpkins, 2001).

In unweathered aquitards, solute transport is controlled mostly by diffusive processes but advection can sometimes be significant (McMahon *et al*., 1999; Robertson *et al*., 1996). In one relatively intact Canadian silt-rich till, agricultural nitrates penetrate through the brown (oxidised) till near the surface but are not detected in the grey (reduced) till deeper down, despite the tritium pulse penetrating much deeper which infers nitrate attenuation (Robertson *et al*., 1996). The redoxcline, and hence the nitrate pulse, was calculated to be moving downward at approximately 1 mm per year as opposed to the tritium pulse, moving at 160 to 200 mm per year. Haloes of oxidised clay around macropores and small fissures are also commonly identified below the redoxcline, where bypass flow has transported nitrate or dissolved organic carbon (Jørgensen and Frederica, 1992; Jørgensen *et al*., 2004).

Groundwaters with excess nitrogen have been sampled from the chalk of North East Norfolk, indicating an impact of denitrification (Feast *et al*., 1998). However, isotopic signatures of nitrate in chalk groundwater were not indicative of significant denitrification. This was resolved by postulating that denitrification occurs as waters infiltrate through glacial till deposits before reaching the chalk. The study by Foster *et al*. (1985) also suggests that the superficial deposits of Norfolk contain viable denitrifying bacteria, while the absence of detectable nitrate, but presence of the tritium pulse, suggests that these deposits contribute to denitrification of recharge waters to the chalk. Glacial tills in the UK tend to contain significant amounts of disseminated pyrite and organic carbon, which can act as electron donors for the denitrification process, so these may locally provide some protection to underlying aquifers.

At the aquifer-aquitard interface, McMahon *et al*. (1999) showed how nitrate can diffuse from groundwater in an aerobic aquifer into pore water of an underlying, organic carbon-rich shale, where the nitrate is reduced. It is suggested that the denitrification rate is probably controlled by the rate of nitrate diffusion across the interface, rather than reaction kinetics.

5.5 Groundwater – surface water interface

There is growing interest in the use of riparian zones and wetlands (natural and constructed) as buffers to surface water bodies from non-point source pollutants such as nitrates. These are characterised by dynamic zones of horizontal and vertical heterogeneity, where reducing conditions and near-constantly saturated sediments high in labile organic carbon facilitate denitrification (Cirmo and McDonnell, 1997; Hill, 1996). Following a brief introduction to the terminology, the next sub-sections discuss conditions favourable for denitrification at the margins of surface water bodies. These can arise because of their position in the landscape, by geological heterogeneity, by the distribution of organic carbon in the sub-surface, or though seasonal influences. Succeeding sub-sections discuss the particular cases of the hyporheic zone and marine fringes.

5.5.1 Hydrogeological and hydrochemical characteristics

The groundwater–surface water interface can be conveniently divided into two or three zones, which, although they share some general qualities in terms of hydrogeology, hydrochemistry, biodegradation potential and vegetation, also have notable unique qualities. These are the riparian zone, riparian wetlands and the hyporheic zone (Figure 5.5); for the purposes of this report these three zones are loosely defined as follows.

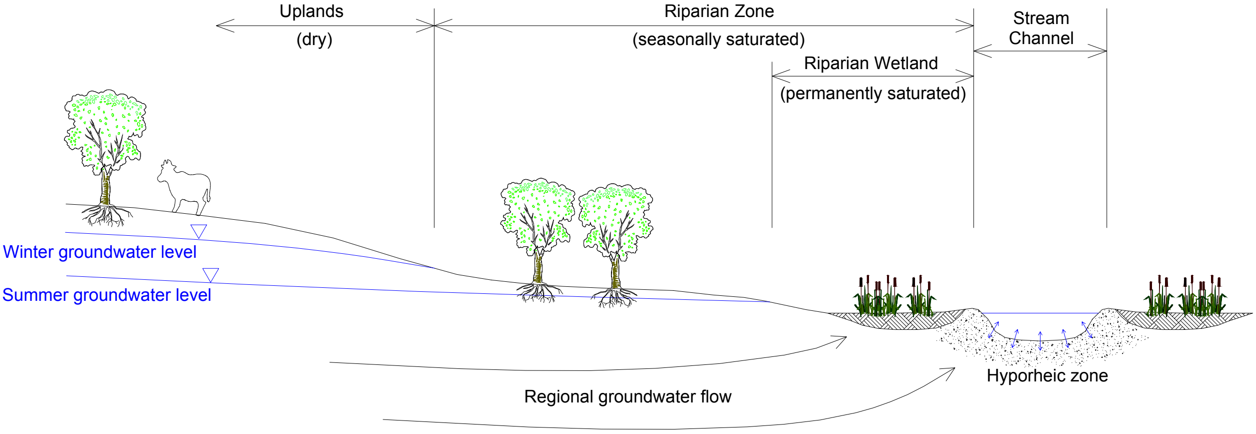


Figure 5.5. Zonation of groundwater wetness within a typical aquifer/river transition zone

Riparian zones

The riparian zone is the area adjacent to a stream or river that is dependent on a variably moist regime. There is clearly overlap between what may be considered a riparian zone, and what may be considered a shallow permeable aquifer (Section 5.3). The key difference between these is the elevation of the water table during seasonal fluctuations: in a riparian zone, the water table is expected to reach the soil zone only during the wetter months, whereas in a shallow aquifer the soil would rarely be saturated. Water, organic matter and nutrients are imported when surface water flooding inundates the riparian zone (Naiman and Decamps, 1997).

Riparian wetlands

In general, riparian wetlands are areas associated with watercourses in which the water table is at or near the surface most of the year. They therefore develop soils that are anoxic from long periods of, or constant, saturation. Vegetation develops adapted to wetland conditions, particularly the lack of soil oxygen (Keddy, 2000). However, as wetlands tend to blend gradually into the riparian zone, they may often be classified as part of the riparian zone, and in the literature this distinction is often not made.

Hyporheic zone

The hyporheic zone is the water-saturated region below and adjacent to a surface water body, which is the interface between a surface water body and groundwater. It is the zone in which groundwater and surface water mix. Typically in the UK it extends no more than a metre vertically below the river and a few metres laterally beyond the river margins, but hyporheic fauna have been identified ten metres beneath the river bed and more than a kilometre from the margins of one river with a wide alluvial plain (Stanford and Ward, 1988). Flow of water within the hyporheic zone is complex and often localised (Conant Jr., 2004; Malcolm *et al*., 2002). High interstitial solute concentrations (Sheibley *et al*., 2003) and large DOC flux (Sobczak and Findlay, 2002) make the hyporheic zone biologically very active. The detailed hydrogeology and hydrochemistry of the hyporheic zone is discussed further in Section 5.5.7 and in Environment Agency 2005.

5.5.2 Position in the landscape

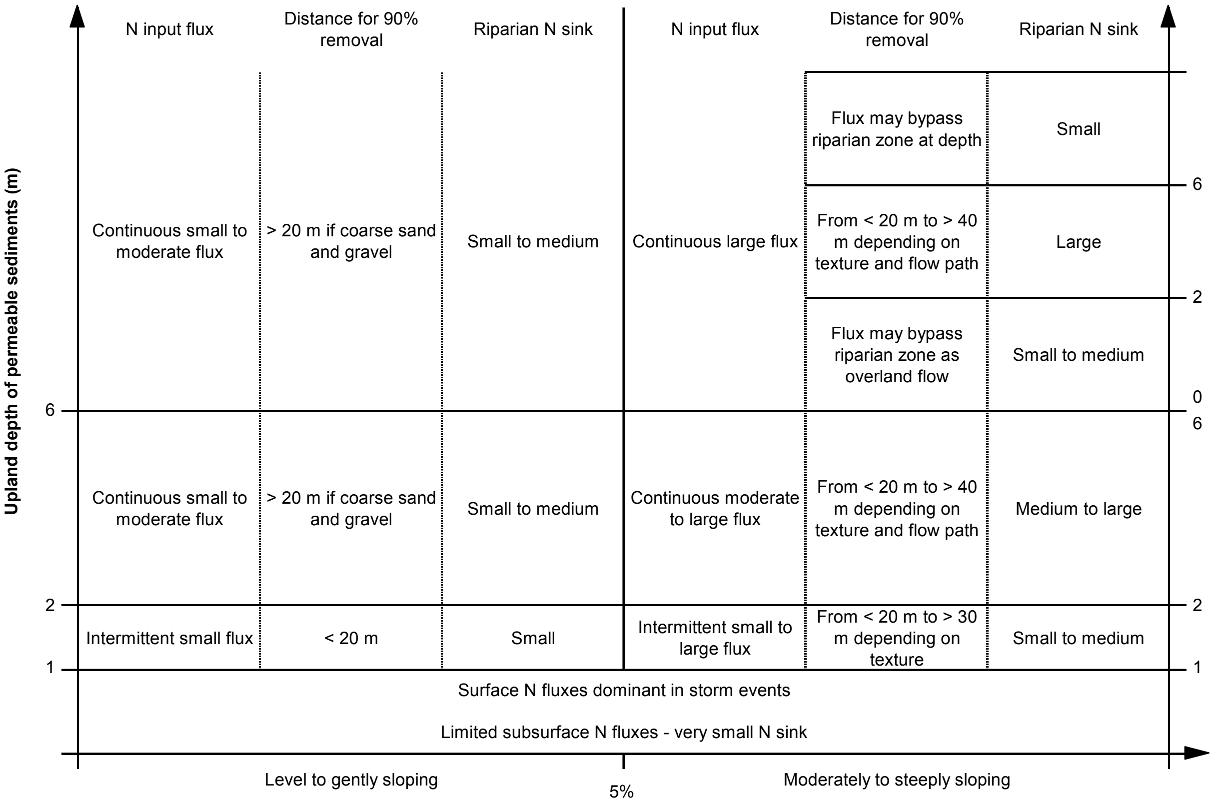
Riparian zones, wetlands and hyporheic zones are strongly influenced by their position in the larger topographic and hydrogeological environment. Position in the landscape will determine some of the inherent local conditions and affect nitrate levels by controlling surface and groundwater delivery, nutrient fluxes and local groundwater flows. Upland aquifer size affects the amount of groundwater flow to the riparian zone and the magnitude of water table fluctuations. Large, deep aquifers upgradient of the riparian zone can significantly decrease nitrate concentrations by dilution with older, nitrate-poor waters (Spruill and Galeone, 2000; Schoonover and Williard, 2003). Local hydrology also plays a part in negating any potential for denitrification in a riparian zone, where drainage ditches can bypass the riparian zones and discharge nitrate-laden runoff or interflow directly into water bodies (Puckett, 2004; Vellidis *et al*., 2003).

5.5.3 Geological heterogeneity

The groundwater–surface water interface is a complex environment that is spatially heterogeneous in both horizontal and vertical dimensions with respect to hydrology, sediment lithology, geochemistry and hydrochemistry (Hill, 1996). The depositional environment of river alluvium is very variable, creating unique and quite often complex interbedded matrices of clay, silt and sand. These develop permeability contrasts, differing organic carbon contents, cracks and fissures, and surface topography. This section discusses the effects of geological heterogeneity on the flow regimes within the groundwater–surface water interface.

Denitrification and carbon degradation both require some residence time in an aquifer, which is influenced in part by sediment permeability. Geologically heterogeneous alluvial deposits therefore potentially act as both a conduit and barrier to groundwater flow. At the widest scale, a generally permeable alluvial deposit favours slow sub-surface flow, allowing for attenuation of nitrate under the right conditions, whereas low permeability alluvium would deflect groundwater via a lower, more permeable aquifer or across the surface, limiting attenuation (Burt *et al.*, 1999; DeVito *et al.*, 2000; Puckett, 2004). On the other hand, discharge via a shallow, thin aquifer underlain by a good aquitard will accentuate the amount of attenuation as all the groundwater flow moves through the shallow zone (Hill, 1996).

A study of denitrification in eight riparian areas in southern Ontario reinforced these views. Vidon and Hill (2004) found that the distance over which nitrate attenuation occurred related directly to the permeability, thickness and slope of the alluvial sediments. The thickness of upland permeable sediments and the slope at the upland margin of a riparian zone were found to influence the magnitude and seasonality of nitrate inputs to riparian zones. The texture and thickness of the riparian zone sediments influence the effectiveness of the riparian zone as a nitrate sink. Figure 5.6 presents the matrix that was developed to explain the conceptual model. It is notable that with a very thin riparian aquifer, most nitrate flux bypasses the zone of active denitrification as overland flow; yet with a thick aquifer, the flow can pass beneath that zone. For any given site, there appears to be an optimum thickness of sediments that will accentuate denitrification.



Upland depth of permeable sediments (m)	N input flux	Distance for 90% removal	Riparian N sink	N input flux	Distance for 90% removal	Riparian N sink	Riparian depth of permeable sediments (m)
	Level to gently sloping			Moderately to steeply sloping			
> 6	Continuous small to moderate flux	> 20 m if coarse sand and gravel	Small to medium	Continuous large flux	Flux may bypass riparian zone at depth	Small	> 6
					From < 20 m to > 40 m depending on texture and flow path	Large	2–6
					Flux may bypass riparian zone as overland flow	Small to medium	0–2
2–6	Continuous small to moderate flux	> 20 m if coarse sand and gravel	Small to medium	Continuous moderate to large flux	From < 20 m to > 40 m depending on texture and flow path	Medium to large	2–6
1–2	Intermittent small flux	< 20 m	Small	Intermittent small to large flux	From < 20 m to > 30 m depending on texture	Small to medium	1–2
< 1	Surface N fluxes dominant in storm events Limited subsurface N fluxes - very small N sink						< 1

Figure 5.6. Conceptual model linking the upland depth of permeable sediments, the riparian depth of permeable sediments, topography and the riparian soil texture to degree of nitrate removal in riparian zones (from Vidon and Hill, 2004).

A heterogeneous alluvial deposit will contain a mixture of both high and low permeability sediments, and flow patterns within the deposit will be complex (Klingbeil, 1998). At a small scale, flow patterns within the alluvial sediment may permit bypass (via preferential flow along gravel lenses) or concentration (via shallow aquitard horizons) of nitrate-rich groundwater through zones of greater denitrification potential (LaMontagne, 2001; Vindon and Hill, 2004). Cey *et al*. (1999) give an example of denitrification on a very small scale, where a plume of nitrate is depleted on passing slowly through a thin (~1 m) layer of clay within a generally sandy alluvium, whereas chloride passes through unattenuated. Figure 5.7 shows the groundwater flow and nitrate concentrations beneath the riparian zone; elevated chloride is present throughout all of Zone 1 in the lower section, whilst nitrate concentrations are clearly depleted as groundwater flows through the clay layer.

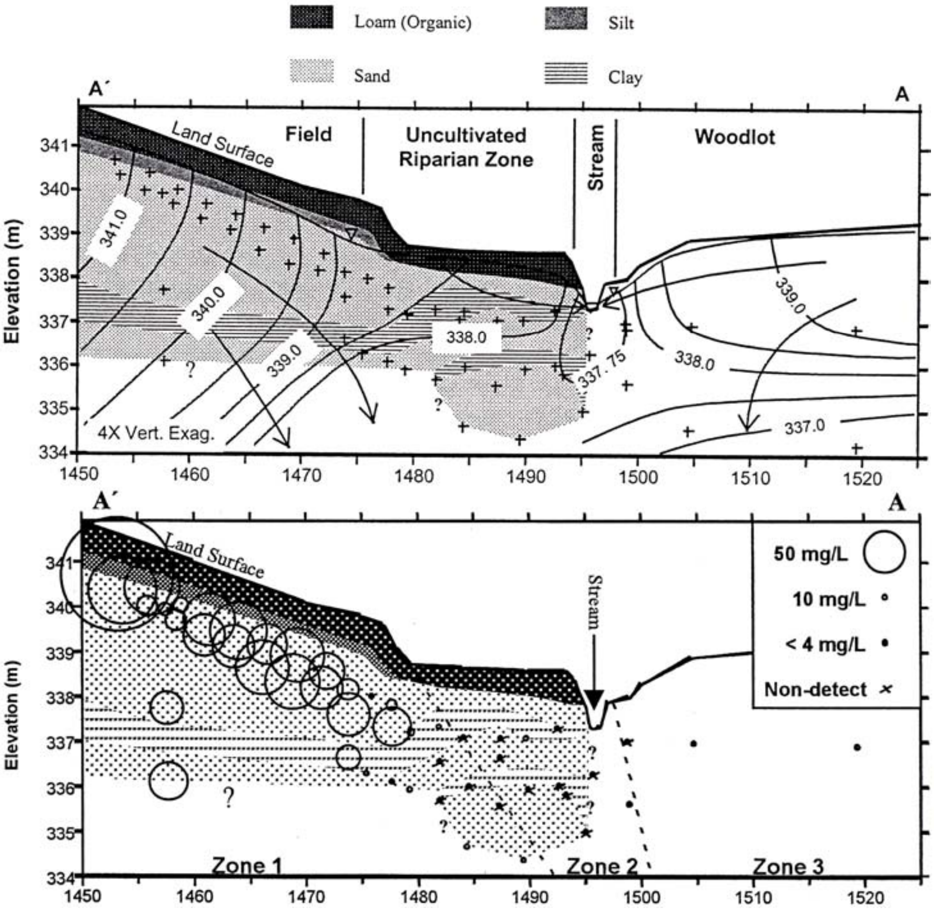


Figure 5.7. Cross sections through a riparian zone (Cey *et al*., 1999). Upper section shows groundwater isopotentials (m) and indicative flow directions. Lower section presents groundwater nitrate concentrations (as NO_3).

5.5.4 Distribution of organic carbon

Although riparian zone denitrification has sometimes been associated with the oxidation of pyrite (Tesoriero *et al*., 2000; Böhlke *et al*., 2002), it is the amount of organic carbon present that makes this such an effective area for denitrification. The carbon is replenished by natural leaf fall, plant die-off, root turnover and root exudates, although inputs are seasonal (Section 5.5.6). Carbon fluxes also relate to the vegetation type: those with high biomass, when dying off, will contribute more degradable carbon, as well as providing a larger, deeper and finer root network. For example, in western France, Clément *et al.* (2003) found higher nitrate depletion rates under grass wetlands than shrub dominated sites and, in southern England, Haycock and Pinay (1993) found higher attenuation rates under poplar (99 per cent) than grass (84 per cent). These rate differences are considered more attributable to denitrification driven by availability of the electron donor, than to plant uptake.

As for non-riparian soils and sub-soils (Section 5.1.2), denitrification potential decreases rapidly with depth in riparian zone soils. Burt *et al*. (1999), for example, found that 60 per cent of the potential denitrification at a riparian site in Oxfordshire occurred within the top 10 cm of the soil column; little activity was identified beneath 40 cm depth. A relationship between organic carbon content and denitrification potential was not derived in this instance.

In addition to the decrease in organic carbon with depth through the unsaturated zone, carbon content varies considerably in the horizontal dimension. In vegetated areas, carbon will be locally replenished by the degradation of plant materials, while surface waters provide organic carbon to riparian zones through deposition during flooding. Deposition is highly non-uniform and can create localised areas of high and low denitrification potential (Jacinthe *et al*., 1998; Martin *et al*., 1999). Ontario Hill *et al*. (2000) found that denitrification occurred in a sandy alluvium as the water moved from sand into lenses of peat or clay. However, the main contribution to bulk denitrification capacity of the riparian zone was from patches of sedimentary organic material within the sands which showed little decrease in permeability and therefore groundwater throughput. Jacinthe *et al*. (1998) and Hill *et al*. (2000) found that the denitrification rate in sediments with patchy carbon distribution correlated well with organic carbon content. However, despite significant spatial variation in dissolved organic carbon and total carbon beneath a riparian forest in Rhode Island, Groffman *et al*. (1996) observed very little corresponding variation in denitrification rate over the 10 m scale, though there may have been more heterogeneity at smaller scales.

The quantity of available organic material in a wetland may be sufficient to drive DNRA (Section 4.9.1) rather than denitrification. This is not covered in great detail in the literature, and only appears to occur in a limited number of cases (Tobias *et al*., 2001).

5.5.5 Uptake by vegetation

In this section uptake by riparian vegetation alone is reviewed; grass or crops are not discussed. Although crops and grass will be responsible for significant uptake of nitrogen and nitrates, this occurs at the source rather than the discharge area, so affects the source term rather than attenuation. Plant uptake may contribute significantly to nitrate attenuation in riparian zones, though it may be difficult to differentiate between plant uptake and denitrification and few researchers have attempted to do so. Those who have, however, find plant uptake less significant (Cey *et al.,* 1999; Clément *et al*., 2003; Hinkle *et al*., 2001; Hill, 1996), suggesting that microbial denitrification is probably the dominant attenuation process in most riparian areas. However, Clement *et al*. (2003) and Schoonover and Williard (2003) found that plant uptake was at least seasonally important.

Studies of vegetative growth in riparian forests have found that biomass increases where groundwater nitrate inputs are higher, but this reduces significantly with the age of the forest, and is most significant with vegetation less than 30 years old (Hill, 1996). This might be due partly to the mineralisation of in-situ nitrogenous organics in the aquifer matrix. An experiment under a poplar forest seems to have identified plant uptake of nitrate as significant: in a constructed riparian forest, with one and two trees per block of forest, 11 and 14 per cent more nitrate was removed respectively compared with areas without trees (Hill, 1996). Again, this may in part be due to increased carbon inputs from root biomass and exudates, though uptake is probably a significant factor.

5.5.6 Seasonality

Notwithstanding the seasonal variability of nitrate inputs to groundwater (Section 2.3), the extent of nitrate attenuation is seasonal, especially in riparian zones, because of fluctuation in water table depth, varying inputs of nitrate and organic carbon, and the plant growth cycle. The following section discusses these aspects.

The elevation of the water table at a riparian site is controlled by the average level of the surface water body and the amount of recharge that has occurred in the catchment up gradient; consequently, water tables are higher in the winter. Seasonal fluctuations in the elevation of the water table regulate the anaerobic capacity of soils and horizons through which groundwater flows (Burt *et al*., 1999; Simmons *et al*., 1992).

Carbon inputs vary with season according to the growth cycle of vegetation and the amount washed through by infiltration, flood waters or snow melt. Root exudates are a significant source in the summer, whilst plant die-off and decomposition of leaf litter are highest in autumn. The elevation of the water table, and therefore the thickness of the unsaturated zone, also affects the amount of mineralisation of organic matter as it infiltrates; as a consequence, DOC levels tend to be higher in winter. Nitrate levels are also higher in winter: since plant uptake is minimal, the high infiltration washes nitrates down to the groundwater (Burt *et al*., 1999).

These factors indicate that denitrification activity in riparian wetlands is likely to be highest in the winter months, and the references cited above have found that to be the case. However, in western France. Clement *et al*. (2003) detected the opposite relationship, where in two riparian wetlands denitrification was the principal sink of nitrate in summer (August). In spring (April) and winter (February), when groundwater levels were within the rooting zone, plant uptake was the dominant sink of nitrate. Schoonover and Williard (2003) found the same result for two riparian zones in Illinois. In Rhode Island, Groffman *et al*. (1996) also found higher denitrification rates in summer (June and September) than winter (March and February) in a deciduous riparian forest, with the availability of organic carbon being the rate-controlling factor.

5.5.7 Processes in the hyporheic zone

A hyporheic zone in contact with a surface water body is permanently saturated, with the direction of transfer between groundwater and surface water being controlled by local hydraulic gradients and the topography of the stream bed. With relatively homogeneous bed sediment the flow patterns can be simple, whether the river is gaining or losing. With heterogeneous sediments or stream bed topography, flow components include the following for a gaining section of channel (Fraser *et al*., 1996) (Figure 5.8):

a) water recharged vertically into the stream bed or laterally into stream banks from common bed forms, such as gravel bars, riffles and debris. Figure 5.8 schematically shows the surface water entering these features upstream and exiting downstream;

b) convective flow resulting from localised pressure variations on the sediment surface. Fluids develop a pressure gradient between slow and fast-moving regions. These may be set up between deep and shallow regions of the stream, for example;

c) water drawn by capillary action through the sediment by evaporation at the sediment-air interface on the bank;

d) groundwater exchange with the active channel as part of local or regional flow networks.

The surface of a stream bed is a heterogeneous environment and the patterns of discharge and recharge can be very complex at fine (< 10 m) spatial scales and over the course of hydrological events (Conant Jr., 2004; Malcolm *et al*., 2002). Cracks and fissures in the sediment can speed up flow, possibly preventing attenuation by reducing time in biologically active sediments or diverting groundwater around the active area of attenuation altogether (such as via springs) (Conant Jr., 2004).

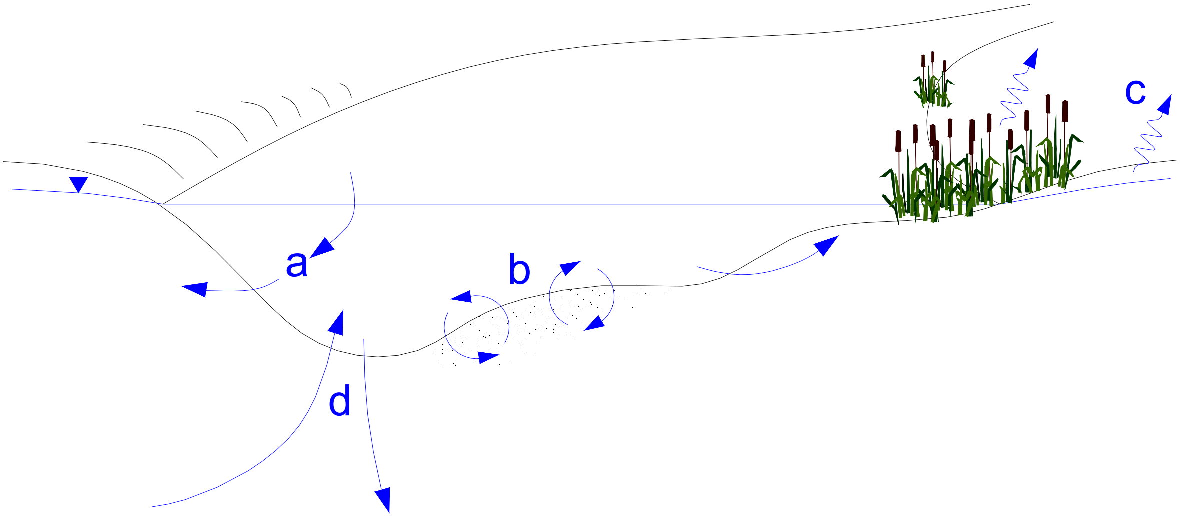


Figure 5.8. Mixing and flows within a hyporheic zone

Biological and physical processes within the hyporheic zone may significantly modify the chemistry of the interstitial water and/or attenuate the movement of solutes. Hyporheic exchange, or movement of surface water into and out of the stream bed, increases hydrologic retention time and the volume of water in contact with sediment biota (biofilms), enhancing biological reactions. The interstitial water of the hyporheic zone is often enriched with nutrients relative to either surface or groundwater, including inorganic nitrogen, DOC and phosphorus (Sheibley *et al*., 2003 and references cited therein). DOC flux through the hyporheic zone is high and is constantly replenished by stream-borne DOC (although with seasonal variability –Chapman *et al*., 2005), and large stocks of particulate organic debris (from riparian zone vegetation or upstream) provide an additional carbon source (Sobczak and Findlay, 2002).

Many studies report denitrification of groundwaters and surface waters within the hyporheic zone (in France: Doussan *et al*., 1997; Grimaldi and Chaplot, 2000; Baker and Vervier, 2004; in North America: Hinkle *et al*., 2001; Fraser *et al*., 1996; Sheibley *et al*., 2003; McMahon and Böhlke, 1996). However, denitrification is only one route through which inorganic nitrogen may be lost in a hyporheic zone. Others include plant uptake at the stream margins or by in-stream aquatic plants, coupled nitrification-denitrification reactions that oxidise NH_4^+ to N_2O or N_2, or consumption by the in-channel biota (Fraser *et al*., 1996 and references cited therein).

Denitrification in a hyporheic zone still requires anaerobic conditions to develop, so before it can commence, the dissolved oxygen must either be eliminated from influent stream water by the oxidation of organic carbon (Sobczak and Findlay, 2002), or locally anaerobic conditions must develop within biofilms or pore space. Hence redox gradients still occur, albeit over short distances, within the hyporheic zone (Hinkle *et al*., 2001; Boulton *et al*., 1998). As in riparian zones, areas of high denitrification may be patchy; their distribution is controlled by the stream bed topography and locations where organic debris has accumulated, thus causing anaerobic pockets of high DOC (Duff and Triska 2000). Hinkle *et al*. (2001) and Sheibley *et al*. (2003) note that the hyporheic zones of two rivers contain discrete regions in which nitrogen transformations occur. In the zone immediately adjacent to the river, ammonium is converted to nitrogen via a coupled nitrification-denitrification reaction. Further from the river bed, where the groundwater influence is strongest, groundwater nitrates are denitrified; nitrogen cycling processes therefore appear to be particularly sensitive to shifts in local redox conditions. Grischek *et al.* (1998) found that the extent of denitrification in a river-bank infiltration site (on the River Elbe, Germany) was dependent on river water temperature. In the summer, the high temperatures promoted denitrification immediately in the river bed sediments. However, in winter, nitrate temporarily increased at some sample locations from nitrification of ammonium in the river bed sediments. Saunders and Kalff (2001) also note a temperature dependence of the denitrification rate in a Canadian lake. This, along with the decreasing organic carbon concentration with depth, were probably the main reasons that that thin littoral zone of the lake was responsible for most of the denitrification.

The geological conditions of the hyporheic zone are also critical to the degree of nitrate attenuation. Grimaldi and Chaplot (2000) found that while nitrates were depleted in a stream bounded by sandy peat on granite (as were nitrates in influent groundwater), no attenuation was observed where the stream was bounded by low-permeability loam on schist. It was concluded that the low permeability of the loam/schist hyporheic zone limited the input of stream water towards the denitrifying sites and the return of denitrified water towards the stream. However, it is not necessarily the case that high permeability hyporheic zones are conducive to denitrification, given that they promote mixing and exchange with oxygen-rich surface waters. A study of a 7 km urban reach of the River Tame receiving groundwater baseflow from the unconfined Birmingham Triassic sandstone aquifer and overlying permeable sand-gravel deposits predominantly observed the hyporheic zone to be low to moderately oxygen-rich, with conditions only occasionally sufficiently reducing for denitrification (Ellis, 2003).

5.5.8 Marine fringes

A special case of groundwater-surface water transition zone is that of the marine fringe, which also seems to be effective at removing groundwater nitrate under some conditions (Tobias *et al*., 2001). Similar considerations appear to be important in determining whether a particular fringing marsh is effective at denitrification, as whether a fresh water riparian zone is effective. For example, shallow groundwater flow encourages contact with the most active denitrification layers, and a high rate of organic material input encourages anaerobic conditions and provides electron donors. Notably, in situ strains of denitrifying micro-organisms do not seem to be sensitive to saline environments (Section 4.5.6).

Dong *et al.* (2002) show how denitrification occurs in the hyporheic zone of the Colne Estuary, England. At the freshwater end of the estuary, nitrates from the river water are transported to the surface of the organic-rich estuarine muds as the tide comes in. The nitrates diffuse into the muds where they are denitrified; the surfacial layers of the muds are most active. Gaseous nitrogen is not formed by this process; the estuarine muds are a source of N_2O to the environment.

5.6 Permeable reactive barriers

Denitrification has in recent years been used within engineered sub-surface facilities built for groundwater contaminant control and remediation. Permeable reactive barriers (PRBs) have been shown to be very effective at attenuating many varieties of groundwater pollutant (Environment Agency, 2002). Commonly, zero-valent iron (Fe^0) is used to catalyse the reduction of chlorinated hydrocarbons, but alternative barrier materials are increasingly being considered for the remediation of inorganic contaminants (Blowes *et al*., 2000: Smith *et al.* 2003)). Most commonly, the treatment method of choice is the use of an organic carbon amendment that provides excess electron donor, typically wood chippings or sawdust (see references below). Hunter (2003) used soya oil in column experiments.

Organic carbon-based PRBs have been installed in two configurations. Horizontal layers are typically installed beneath new designed sources of nitrate, such as septic system infiltration systems, whereas vertical layers are typically installed downstream of existing pollutant sources (Robertson and Cherry, 1995). An advantage of horizontal installations is that, if they are constructed with suitably fine-grained material, they can permanently retain water by capillary forces and stay anaerobic even when above the water table. Sawdust-amended barriers can remove in excess of 95 per cent of nitrate load, for at least six to seven years of operation (Robertson *et al*., 2000; Schipper and Vojvodić-Vuković, 2000). A common observation is that denitrification rates, even when the source of organic carbon has aged, are not limited by carbon availability but by upstream nitrate concentrations. Without pH control, the denitrification reaction can potentially increase the pH out of the range preferred by heterotrophic bacteria (Rust *et al*., 2000) and the wall may need amendment with an acid buffer.

One consequence of adding dissolved organic carbon to an aquifer is that anaerobic plumes may develop downstream that may be of significant concern if the treated groundwaters discharge to sensitive ecological receptors (MacQuarrie *et al*., 2001b). Another issue when using a sawdust-amended PRB to attenuate nitrate is that, because nitrate plumes from soakaways are often found in permeable formations, the fill material needs to be sufficiently permeable that groundwater does not simply flow around it (Schipper *et al*., 2004).

6 Conclusions

6.1 Discussion and implications for environmental management

Elevated nitrate concentrations in groundwater are a particular concern because of the costs incurred in treating ground and surface waters to meet drinking water standards and the potential risk of eutrophication of surface waters. This report has discussed the mechanisms of nitrate attenuation in the sub-surface, given appropriate hydrogeological or geochemical conditions under which its movement can be retarded, and described the biogeochemical conditions under which it can undergo denitrification to nitrogen or nitrous oxide gas.

Apart from physical attenuation processes such as dispersion, the attenuation of nitrate in groundwater is almost always by denitrification. To summarise Section 4, denitrification activity requires all of the following conditions:

- presence of nitrate;
- presence of denitrifying bacteria;
- presence of a biodegradable electron donor (organic carbon, reduced iron and/or reduced sulphur);
- anaerobic conditions (dissolved oxygen concentrations less than circa 1-2 mg/l; possibly due to the presence of an excess amount of electron donor);
- favourable environmental conditions (such as temperature, pH, other nutrients and trace elements)

The presence of nitrate is assumed. Because denitrifying bacteria appear to be almost ubiquitous in the sub-surface, the critical limiting factors for denitrification are the presence of anaerobic conditions and the presence of a suitable electron donor. In hydrogeological environments where these are present, the other ambient environmental conditions do not seem to be of particular concern, although they are important in artificial denitrifying environments (in other words, N treatment systems).

There is a sound fundamental understanding of the processes controlling nitrate attenuation in the unsaturated soil zone. However, in groundwater relatively little is understood about the geochemical conditions that determine whether denitrification will take place, beyond a broad appreciation that denitrification is unlikely to be significant under well-oxygenated conditions (when dissolved oxygen concentrations are above circa 1-2 mg/l). Similarly, little is understood about the groundwater conditions that control the rate and extent of denitrification. The following sections summarise and discuss the findings of this report.

6.1.1 Physical attenuation mechanisms

Nitrate is largely unretarded by sorption in the sub-surface, is non-volatile and does not biodegrade under aerobic conditions. However, some physical solute transport processes that act upon all solutes do attenuate the movement of nitrate. These include dispersion, advection through the unsaturated zone and dual porosity effects, in particular diffusion into low-velocity matrix groundwater (Section **Error! Reference source not found.**); all act to reduce the velocity of movement and eventual maximum concentration of nitrate. However, they are conservative processes and therefore only act to prolong the duration of the impact at the receptor, albeit at lower maximum concentrations. Prediction of potential solute attenuation by dispersion and advection through the unsaturated zone is a well-documented science (for instance, Fetter, 1999), although the quantifying the constitutive relationships can be difficult and expensive.

6.1.2 Predicting biodegradation in aquifers and aquitards

In the unsaturated zone of the Chalk and Permo-Triassic Sandstones, very detailed studies have demonstrated minor decreases in nitrate concentrations within infiltration water. However, where it has been quantified, the losses are of the order of one to two per cent of the nitrate load in the infiltrating water. It is unlikely that these processes offer an opportunity to significantly impact regional groundwater quality.

In the saturated zone of the Chalk, Permo-Triassic Sandstones and Lincolnshire limestone, denitrification only occurs once the aquifer becomes confined and dissolved oxygen is depleted. The evidence for denitrification is weak because these regional-scale studies have tended to infer denitrification from decreasing nitrate concentrations in a few monitoring points (often abstraction wells) without isotopic data. Although these may well be instances of denitrification, the rates of reaction in the confined zones are found to be slow in comparison to the timescale over which nitrate loads have increased in the last half-century. Nevertheless, major public water supply boreholes are almost always located in the unconfined zones of such aquifers, so in situ denitrification provides no significant protection to public sources.

Except in riparian zones, no UK studies have been identified that illustrate the attenuation of nitrate in shallow groundwater environments. This is probably because the large-throughput septic soakaways studied in Canada and the US are much less common in this country, and because sand and gravel deposits in the UK are on a much smaller scale and are not commonly a source of drinking water. Although the hydrogeological environments and source inputs do vary from the case studies presented, the weight of evidence for shallow systems suggests they exhibit denitrification from both increased natural and anthropogenic carbon inputs.

The wide variety of UK aquitard formations has not been studied in depth (Section 5.3), but denitrification may occur in the Norfolk tills, and the geochemistry and hydrogeology of UK tills and other aquitards are potentially conducive to denitrification (high electron donors such as organic carbon and iron pyrite; low permeability with limited oxygen diffusion and relatively long groundwater residence times).

6.1.3 Groundwater–surface water interface

Riparian zones, wetlands and hyporheic zones appear to be zones of effective nitrate attenuation, primarily because of the high fluxes of organic carbon that can drive denitrification, and saturated conditions near to, or within, the soil zone. Very few UK studies were identified for this topic, so most of those cited here are North American. The similarity of climate and vegetation makes them applicable in the UK, with suitable caveats.

Nitrate attenuation by both plant uptake and denitrification occurs mainly in the biologically active upper parts of the water column, near the soil zone. Hydrogeological conditions which promote shallow groundwater flow are therefore key to determining the extent of attenuation across the riparian zone (Section 5.5.3). If suitable hydrogeological conditions are present, the rate of biodegradation of nitrate in riparian zones is controlled by the availability and reactivity of organic carbon (Section 5.5.4). Seasonal variation in nitrate attenuation is observed via the following processes: the plant growth cycle, depth of the water table, organic carbon inputs and average temperature (Section 4.5.5) control rates of plant uptake and denitrification activity.

In assessing whether a riparian zone might be effective at attenuating nitrate, it is essential to fully characterise both the horizontal and vertical variation in geological and geochemical conditions within the zone. Overall, the prediction of whether nitrate attenuation will occur in a given riparian zone is complex because of the many contributory factors. However, the table from Vidon and Hill (2004) – Figure 5.6 in this document – may prove a useful starting point, given that it combines a number of features of a riparian zone (soil type, hydrogeology and upland catchment size) to assess its effectiveness for attenuating nitrate.

6.2 Identification of knowledge gaps and research needs

There is a sound fundamental understanding of the processes controlling nitrate attenuation in the unsaturated soil zone. However, in groundwater, relatively little is understood about the geochemical conditions that determine whether denitrification will take place, beyond a broad appreciation that denitrification is unlikely to be significant under well-oxygenated conditions (when dissolved oxygen concentrations are above circa 1-2 mg/l). Similarly, little is understood about the groundwater conditions that control the rate and extent of denitrification. Consequently, a better understanding of the geochemical conditions that trigger and sustain denitrification in UK aquifers would help to support risk assessment and modelling of nitrate attenuation.The following studies are recommended:

1. Determine the bioaccessibility of organic carbon in the matrices of the Chalk, Permo-Triassic Sandstone, Lincolnshire limestone and other important aquifers. Determine the factors controlling bioaccessibility, such as type of organic carbon, diffusion of reactants through the sediment matrix and micro-organism movement in the matrix.

2. Determinne the distribution of dissolved organic carbon in confined and unconfined UK aquifers. Again, determine how available it is for microbial reactions. Compare which of the following is more indicative of denitrification potential in UK major aquifers: solid or dissolved organic carbon.

3. Revisit regional-scale studies of the depletion of nitrate concentrations in confined groundwater, taking new samples and using isotopic and other definitive methods to determine the contribution of denitrification.

4. Review recent baseline hydrochemistry studies and other hydrochemical studies as a screening process to identify in which aquifers denitrification might be observed. Examine the relationships between dissolved oxygen, nitrate concentrations and redox potential to prioritise aquifers for more detailed investigation (such as for point 3 above).

5. At catchment scale, determine the relationship between groundwater and surface water concentrations of nitrate. Understand whether differences can be explained using mechanisms other than denitrification in riparian or hyporheic zones.

6. Assess whether the micro- and meso-scale studies of denitrification in fracture networks can be scaled up to regional (aquifer) scale.

7. Use or develop suitable dual porosity modelling tools to understand flow to abstraction boreholes and residence times in aquifers and to determine when the beneficial effects of defining nitrate vulnerable zones might be seen.

For locally contaminated groundwater bodies, there may be circumstances where remediation is desirable to protect drinking water abstractions or surface water receptors. There is consequently a requirement for the development, evaluation and validation of potential remediation techniques for nitrate-contaminated groundwater. Permeable reactive barriers (PRBs) may have particular benefits in this regard and benchmarking of different PRB matrices would be beneficial to the user community.

Where nitrate contamination is regional, lower intensity remediation processes are likely to represent the only economically viable approach. In such cases riparian buffer zones, if shown to be effective, have great attraction. The weakness with such zones is often a failure to achieve effective hydraulic distribution through the active treatment portion of the system. Whilst the achievement of this is likely to be site-specific, guidance on best practice when establishing such buffer zones (including investigation of natural flow regime and potential denitrifying activity and augmentation of natural systems where required) would be a valuable tool.

Glossary of terms

Abiotic	Reaction that takes place without the involvement of biological activity.
Acclimation	The physiological process through which an organism grows accustomed to a new environment. In microbial cultures, this can involve enzymatic changes that allow it to use a new nutrient source for energy. Also termed adaptation.
Adaptation	See acclimation.
Aerobic[1]	An environment containing molecular oxygen; biodegradation or other process operating in the presence of molecular oxygen.
Anaerobic[1]	An environment containing no molecular oxygen; biodegradation or other process operating in the absence of molecular oxygen.
Anoxic[1]	Conditions favouring reductive degradation, for example anaerobic environments.
Aquifer	Saturated underground rock or sediment formation which is sufficiently permeable to allow the flow of water.
Autotrophic	Micro-organisms or metabolic processes in which inorganic carbon compounds are used as the source of carbon for cellular growth and anabolism.
Bioavailability	In situ availability of a chemical to biological processes.
Biodegradation	Biological conversion of a contaminant into simpler compounds.
BTEX	Benzene, toluene, ethylbenzene and xylene.
Denitrification	Anaerobic biological activity utilising nitrate as electron acceptor. The end-product of respiration is usually nitrogen but intermediate formation of nitrite or nitrous oxide may be detected. Also termed nitrate reduction. See Section 4.2.
DRNA	Dissimilatory reduction of nitrate to ammonium. See Section 4.5.8.
Electron acceptor	Substrate that is reduced during respiration. Common electron acceptors used by micro-organisms include oxygen (in aerobic environments) or nitrate, iron (III), sulphate, manganese (IV) (usually in the absence of oxygen).
Electron donor	Substrate that is used in metabolism to supply electrons to the respiratory chain and is hence oxidised. Common electron donors used by micro-organisms include organic carbon, iron (II), manganese (II) or sulphide minerals.
Eutrophication	The loss of trophic conditions, normally as a result of nutrient enrichment of an aquatic system by nitogen or phosphorus.
Fermentation	A redox process in which the organic carbon substrate acts as both electron donor and electron acceptor.
Fermenter	A micro-organism that obtains energy via fermentation of organic substrates.

Ferric iron	Iron (III)
Ferrous iron	Iron (II)
Heterotrophic	Micro-organisms or metabolic processes in which organic compounds are used as the source of carbon and energy for cellular growth and metabolism
Hyporheic zone	The region around a river within which groundwater and surface water mix
Macropore	A large pore in a soil or other porous medium (generally created by root holes, worm holes or desiccation cracks amongst other processes)
Mineralisation	Biodegradation that leads to the transformation of contaminants into inorganic end-products, such as carbon dioxide, water, methane, chloride ions.
Nitrate reduction	See denitrification.
Nitrification	The oxidative conversion of ammonium to nitrate (Environment Agency, 2003).
Oxic[1]	Conditions favouring oxidative degradation; for example, aerobic environments or those where nitrate is a major microbial respiratory substrate.
Oxidising	Conditions favouring oxidative degradation; for example, aerobic environments or those where nitrate is a major microbial respiratory substrate.
Redox reaction	A reaction which involves the transfer of one or more electrons between molecules. Microbial respiration is a series of redox reactions.
Redoxcline	The boundary in an aquifer where redox conditions rapidly change (usually from oxidising to reducing conditions)
Reducing	Conditions favouring reductive degradation. For example, anaerobic anoxic environments where microbial respiration is generating methane or hydrogen sulphide.
Riparian zone	The area adjacent to a stream or river that is dependent on a variably moist regime.
Sulphate reduction	Anaerobic biological activity utilising sulphate as an electron acceptor. The product of respiration is sulphide, which will normally be detected in groundwater as metal sulphide salts or H_2S.
Unsaturated zone	The zone between the land surface and the water table. It includes the soil zone, unsaturated rock and capillary fringe. The pore spaces contain water at less than atmospheric pressure, as well as air and other gases. Saturated bodies such as perched groundwater may exist within the unsaturated zone. Also called zone of aeration or vadose zone.

[1] See extended discussion of these definitions in Section 1.3.

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Appendix 1. Literature search method

Literature searching was conducted with commercial abstracting sources and internet resources.

The following commercial abstracting services were used:

- Cambridge Scientific Abstracts
- ISI Web of Science

Internet resources used included:

- www.sciencedirect.com
- www.scirus.com
- www.epa.gov
- www.usgs.gov
- www.google.co.uk
- scholar.google.com
- www.bgs.ac.uk
- www.ingenta.com

The keywords used for the searches were generally as follows

Keyword = nitrate AND (attenuation or degradation) AND (groundwater or "ground water" or hyporheic or riparian or aquifer)

Keyword = (denitrification or denitrifying or "nitrate reduction") + (groundwater or "ground water" or hyporheic or riparian or aquifer) + not (hydrocarbon or oil or solvent or chlorinated)

Keyword = nitrate + plume + (groundwater or "ground water" or aquifer) + not (hydrocarbon or oil or solvent or chlorinated)

Keyword = (nitrate or nitrite) AND (sorption or retention) AND (groundwater or "ground water" or hyporheic or riparian or aquifer)

We welcome views from our users, stakeholders and the public, including comments about the content and presentation of this report. If you are happy with our service, please tell us about it. It helps us to identify good practice and rewards our staff. If you are unhappy with our service, please let us know how we can improve it.

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