Review

Nitrate attenuation in groundwater: A review of biogeochemical controlling processes

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ARTICLE INFO

ABSTRACT

Biogeochemical processes controlling nitrate attenuation in aquifers are critically reviewed. An understanding of the fate of nitrate in groundwater is vital for managing risks associated with nitrate pollution, and to safeguard groundwater supplies and ground-water-dependent surface waters. Denitrification is focused upon as the dominant nitrate attenuation process in groundwater. As denitrifying bacteria are essentially ubiquitous in the subsurface, the critical limiting factors are oxygen and electron donor concentration and availability. Variability in other environmental conditions such as nitrate concentration, nutrient availability, pH, temperature, presence of toxins and microbial acclimation appears to be less important, exerting only secondary influences on denitrification rates. Other nitrate depletion mechanisms such as dissimilatory nitrate reduction to ammonium and assimilation of nitrate into microbial biomass are unlikely to be important in most subsurface settings relative to denitrification. Further research is recommended to improve current understanding on the influence of organic carbon, sulphur and iron electron donors, physical restrictions on microbial activity in dual porosity aquifers, influences of environmental condition (e.g. pH in poorly buffered environments and salinity in coastal or salinized soil settings), co-contaminant influences (particularly the contrasting inhibitory and electron donor influences of pesticides) and improved quantification of denitrification rates in the laboratory and field.

Contents

1.   Introduction

Since the 1970s, nitrate (NO$_3$) contamination of groundwater has become a significant environmental problem, with many parts of the world now reporting groundwater nitrate pollution (Burden, 1982; Spalding and Exner, 1993; Beeson and Cook, 2004; European Environment Agency (EEA), 2000; Rao, 2006; Rivett et al., 2007; Roy et al., 2007; Organisation for Economic Co-operation and Development (OECD), 2008). The consequences include long-debated health concerns arising from increased risks of methaemoglobinaemia and cancer (Fan and Steinberg, 1996; WHO, 1999; WHO, 2004; Höring and Chapman, 2004), and environmental impacts such as the eutrophication of surface waters due to excess nutrients (Vitousek et al., 1997; WHO, 1999; Mason, 2002). Diffuse pollution from intensive agriculture since the mid-20th century has largely been blamed for these problems (Foster, 2000). It has been estimated that 70–80% of the nitrate in English surface and groundwaters is derived from agricultural activities (Defra, 2002). However, direct application of nitrogen-based fertilizers to land is not the only source. Discharge from septic tanks and leaking sewers, atmospheric deposition and the spreading of sewage sludge and manure to land can all contribute (Wakida and Lerner, 2005).

The European Union and World Health Organization (WHO) have both set the standard for nitrate in potable water at 11.3 mg nitrogen (N) per litre (50 mg-NO$_3$/l) (Drinking Water Directive 98/83/EC; WHO, 2004). The cost of compliance is already significant (Knapp, 2005). In the UK alone, the cost of treatment to ensure potable water supplies are below 50 mg-NO$_3$/l amounted to £16 million per annum during 1992–1997 (Dalton and Brand-Hardy, 2003) and is predicted to rise to £58 million per annum by 2010 as low-nitrate water for blending becomes scarcer (Defra, 2006).

The European approach to the problem has increasingly recognized the need for integrated protection and management of water resources. The Nitrates Directive (91/676/EEC) requires protection of all natural freshwaters and sets a limit of 50 mg-NO$_3$/l which applies to all groundwater irrespective of its intended use, though it is recognized that much lower N concentrations, possibly around 4.4–8.8 mg-NO$_3$/l (1–2 mg-N/l), may trigger eutrophication in nutrient-poor (oligotrophic) surface waters (James et al., 2005). In more nutrient-rich waters, phosphorus concentrations are more commonly limiting. In addition, the Water Framework Directive (2000/60/EC) requires all groundwater bodies to achieve good status by 2015. The goal of good status includes a test that groundwater concentrations do not exceed statutory limits, including that set by the Nitrates Directive.

The severity of the nitrate problem is illustrated by European Environment Agency (EEA) data on groundwater nitrate concentrations across the EU (EEA, 2007). The proportion of groundwater bodies with mean nitrate concentration >25 mg-NO$_3$/l in 2003 was reported as being ca. 80% in Spain, 50% in the UK, 36% in Germany, 34% in France and 32% in Italy. Notable exceptions were the Scandinavian and Baltic states where less than 3% of groundwater bodies had reported mean nitrate >25 mg-NO$_3$/l. Variably elevated nitrate concentrations are reported elsewhere, for example, Australia (Australian State of the Environment Committee, 2001) and North America (Hudak, 2000; Hinkle et al., 2007).

Mitigation is difficult due to the long-term, diffuse and continuing nature of the problem (Hiscock et al., 2007; Mathias et al., 2007). Options for alleviation are primarily continued implementation of land-use control measures, such as protection zones designed to reduce subsurface nitrate loading (see for example, Silgram et al., 2005; Hiscock et al., 2007; Johnson et al., 2007), and reliance on natural attenuation processes. Our focus is the latter.

We critically review the scientific understanding of biogeochemical processes that control nitrate attenuation in the subsurface and, specifically, consider processes in the unsaturated zone and groundwater environments (soil zone processes such as plant uptake are excluded). Evidence for retardation of nitrate in groundwater has not been identified, though the process has been observed in some soils due to the presence of poorly crystalline materials that carry variable surface charge, and therefore adsorb otherwise inert anions such as nitrate and chloride (Katou et al., 1996; Clay et al., 2004).

Disregarding retardation, processes that cause nitrate mass removal control the attenuation of nitrate. Denitrification is generally recognized as the most significant mass removal process (Korom, 1992; Burt et al., 1999) and is the primary attenuation process evaluated here. We consider the transformation products generated, the roles of the various electron donors and the effects of environmental conditions. The latter includes assessment of the influence of nitrate and oxygen concentration, nutrient availability, pH, temperature, salinity, toxins, pore size and microbial acclimation. The focus is upon denitrification occurring under natural groundwater conditions and as such reference to denitrification-based water treatment studies is typically excluded, particularly where treatment conditions discussed may not occur naturally (e.g. Schnobrich et al., 2007). Where groundwater-specific examples have not been identified, examples of processes in soil are typically used in analogy. We also discuss nitrate depletion mechanisms other than denitrification such as dissimilatory nitrate reduction to ammonium and assimilation of nitrate into microbial biomass.

Our scope is restricted to a critical review of the biogeochemical processes controlling nitrate attenuation. We summarize the science on biogeochemical processes controlling nitrate attenuation potential at any given locality and indicate gaps in the literature worthy of further research. As such the review is distinct, but complementary, to other reviews. These include the well-cited, but now dated, reviews on denitrification microbiology (Knowles, 1982), saturated zone denitrification (Korom, 1992), natural and artificial denitrification by Hiscock et al. (1991) and tracking denitrification (using ^{15}N abundance) by Mariotti (1986). They also include the more recent reviews of non-agricultural sources of groundwater nitrate by Wakida and Lerner (2005), denitrification occurrence across landscapes and waterscapes by Seitzinger et al. (2006), riparian zone nitrogen removal effectiveness by Mayer et al. (2006) and Haycock et al. (1997), methods for measuring denitrification by Groffman et al. (2006) as well as other case study based reviews such as Kinniburgh et al. (1999), Rivett et al. (2007) and Domagalski et al. (2008) who examine field evidence for denitrification within specific aquifer settings.

2. Denitrification: overview

2.1. Electron acceptor context

Bacteria in aquifers obtain energy from the oxidation of organic compounds or inorganic species (e.g. FeS_2, Fe^{2+}, Mn^{2+}). Bacteria that use organic carbon as their energy source also tend to use it as a source of cellular carbon (heterotrophism), while those that use inorganic compounds normally use inorganic carbon (mainly from HCO_3^-) for cell construction (autotrophism). Bacteria obtain their energy by mediating chemical reactions typically involving intercompound electron transfer.

Fig. 1 shows the fate of organic matter (typically the most common electron donor in aquifers) in the presence of a variety of electron acceptors that commonly occur in the subsurface. Organic carbon tends to be oxidized preferentially with the electron acceptor that supplies most energy to the microorganisms, namely free oxygen (O_2). With an excess of organic carbon, aerobic bacteria use dissolved oxygen until it is depleted whereupon reduction of other electron acceptors becomes energetically favourable. Once oxygen is consumed,

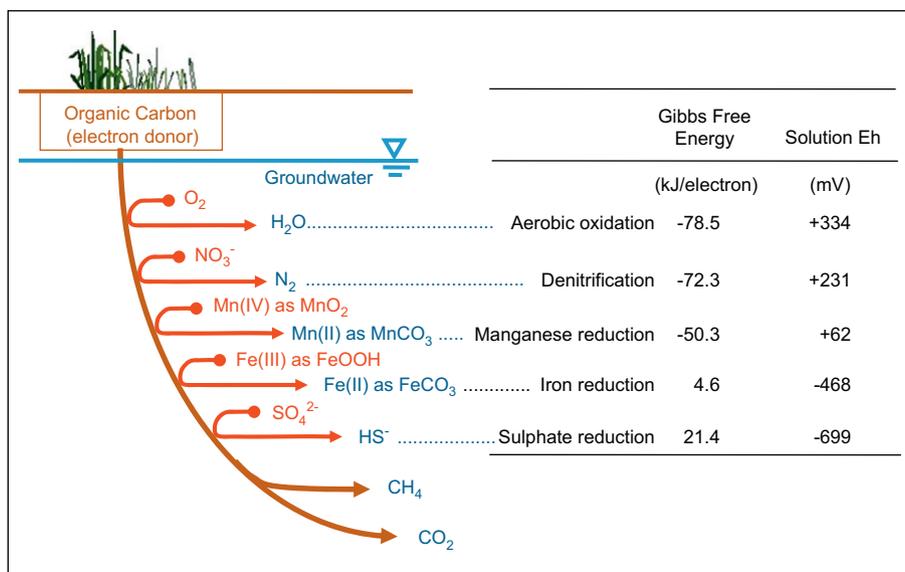|		Gibbs Free Energy	Solution Eh
		(kJ/electron)	(mV)
H_2O...................................	Aerobic oxidation	-78.5	+334
N_2	Denitrification	-72.3	+231
Mn(II) as $MnCO_3$	Manganese reduction	-50.3	+62
Fe(II) as $FeCO_3$	Iron reduction	4.6	-468
HS^- ....Sulphate reduction		21.4	-699

Fig. 1 – Thermodynamic sequence of electron acceptors for oxidation of organic carbon in the saturated zone (adapted from Korom, 1992).

facultative anaerobes (bacteria capable of growing with or without oxygen) use nitrate as an electron acceptor. As oxygen levels decrease, further obligate anaerobes (bacteria that survive only in the absence of oxygen) begin to use the remainder of the available electron acceptors. As nitrate becomes depleted, reduction reactions generally proceed through manganese and iron oxides, then sulphate, and then hydrogen and carbon dioxide, until finally generating methane.

This redox reaction sequence is commonly seen along groundwater flow lines in aquifers (Edmunds et al., 1982) and in landfill leachate plumes (Christensen et al., 2000). In practice, systems seldom exhibit strict redox zone boundaries as a number of redox reactions may occur simultaneously in any single aquifer block (McGuire et al., 2002). Likewise, it is unlikely that groundwater will be at equilibrium with respect to redox and that spatially complex geochemical conditions will prevail (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. Established biofilms can locally control redox conditions and allow a range of redox processes within micro-niche sub-environments that would otherwise not occur at that location (Yu and Bishop, 1998; Bishop and Yu, 1999).

2.2. Denitrification process

Denitrification is central to the nitrogen cycle illustrated in Fig. 2 with respect to the subsurface groundwater environment and involves the reduction of nitrate via a chain of microbial reduction reactions to nitrogen gas (Knowles, 1982).

Nitrate can also be reduced to nitrite and nitrous oxide gas by abiotic reactions but, in the subsurface, these reactions are minor compared with biological denitrification. The organisms capable of denitrification tend to be ubiquitous in surface water, soil and groundwater (Beauchamp et al., 1989). They are found at great depths in aquifers: in clayey sands to 289 m (Francis et al., 1989); in limestone to 185 m (Morris et al., 1988); and in granite to 450 m depth (Neilsen et al., 2006). Denitrifiers are mostly facultative anaerobic heterotrophs and hence obtain both their energy and carbon from the oxidation of organic compounds. However, some denitrifying bacteria are autotrophs, obtaining their energy from the oxidation of inorganic species. In general, the absence of oxygen and the presence of organic carbon, reduced sulphur or iron facilitates occurrence of denitrification.

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

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \tag{1}$$
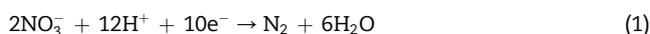
2.2.1. Transformation products

Although denitrification has a stable endpoint at nitrogen gas, the process can be arrested at any of the intermediate stages (Fig. 2). This is important because nitrite is significantly more toxic than nitrate (WHO, 2004) and nitrogen oxides (NO_x) are environmentally destructive gases. The other product of the denitrification reaction is the oxygen rejected at each step. This is typically released as bicarbonate (HCO_3^-) ions, carbon dioxide (CO_2) or sulphate (SO_4^{2-}) ions.
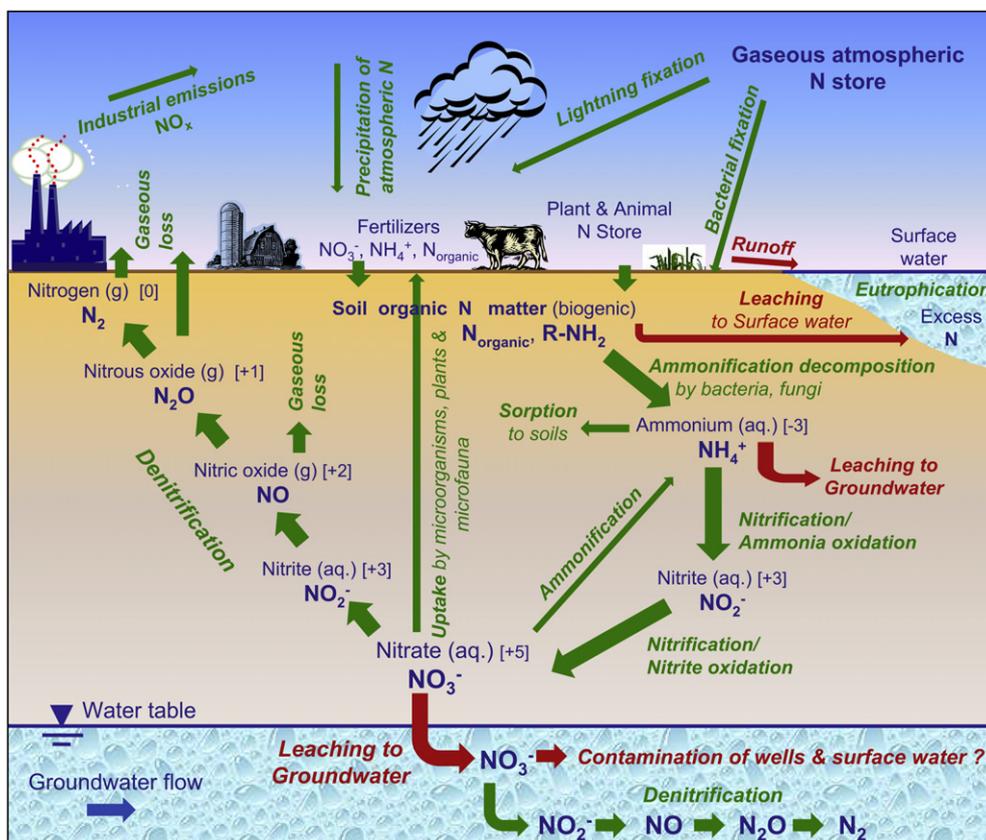

Fig. 2 – The nitrogen cycle and its influence upon the water environment.

Nitrite is significantly more reactive than nitrate and is stable only within a limited range of redox conditions. In particular, the action of the nitrite reductase enzyme is more sensitive to oxygen concentrations than that of nitrate reductase (Hochstein et al., 1984; Korner and Zumft, 1989). The difference in energy available from the reduction reaction means that nitrate is used by denitrifiers preferentially to nitrite, even when both enzymes are present. A build-up of nitrite may then occur due to the time lag between the onset of nitrate reduction and the subsequent onset of nitrite reduction (Betlach and Tiedje, 1981; Gale et al., 1994). In natural waters, nitrite rarely occurs at concentrations comparable with those of nitrate (typically 2–5 orders of magnitude lower; Environment Agency data for 2003), except temporarily under reducing conditions. Nitrite also readily reacts with dissolved organic compounds to form dissolved organic nitrogen compounds (Davidson et al., 2003), especially in low pH environments where nitrous acid (HNO_2) is the key reactant.

Nitric oxide (NO) and nitrous oxide (N_2O) are formed during denitrification but, in favourable conditions, transform rapidly to benign nitrogen gas. Both NO and N_2O 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; this process, rather than denitrification, is the main contributor to N_2O emissions from UK Chalk groundwaters (Hiscock et al., 2003). Free NO is rarely observed because it transforms to N_2O rapidly under typical environmental conditions. It is usually observed only in small-scale laboratory studies as an intracellular intermediate (Scheible, 1993).

When oxygen levels are very low, nitrogen gas (N_2) is the end product of the denitrification process; but, where oxygen levels are more intermediate or variable, the reactions may stop with the formation of NO_x (Brady and Weil, 2002). Very high nitrate concentrations or low pH values also arrest denitrification at the N_2O stage. Indeed, N_2O is often used in wetland studies as an indicator that denitrification is taking place (Bernot et al., 2003; Delaune and Jugsujinda, 2003). Formation of N_2 can be arrested in experimental studies by applying an excess of acetylene (C_2H_2) such that all denitrified nitrogen can be measured as N_2O (Yoshinari and Knowles, 1976). However, the presence of N_2O as an indicator of denitrification is not necessarily conclusive as it may form from partial nitrification of ammonium (Kinniburgh et al., 1999). The denitrification process can be reactivated further along a flow line; for example, LaMontagne et al. (2002) studied an estuarine environment in which groundwater supersaturated with N_2O entered but was converted to nitrogen in anoxic benthic sediments.

At the stable endpoint of the denitrification chain, evolved nitrogen can be obscured by atmospheric nitrogen, especially in shallow systems. However, some studies use the parameter 'excess nitrogen' (i.e. the N_2 concentration above that expected from equilibration with the atmosphere) to identify or quantify denitrification, especially in deep, confined groundwaters (Wilson et al., 1990). For example, Vogel et al. (1981) and Fontes et al. (1991) used such measurements to estimate that denitrification accounted for up to 22 and 46 mg-N/l removal, respectively (very high natural concentrations). See Groffman et al. (2006) for a review on methods for measuring denitrification.

The fate of the oxygen rejected at each step of the denitrification process depends on the electron donor present. If organic carbon is the electron donor, the oxygen forms bicarbonate ions and carbon dioxide; if a sulphide mineral is the electron donor, sulphate ions are formed. Some positive feedback is hence observed in the denitrification process as the production of bicarbonate and carbon dioxide helps to buffer the groundwater pH around the neutral conditions most favourable for the denitrification process. Neutral and basic conditions favour the release of N_2 rather than N_2O.

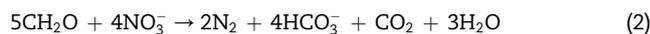### 2.3. Denitrification occurrence in the subsurface

Denitrification in the subsurface environment is controlled by the local biogeochemical conditions (Pinay et al., 1993; Vidon and Hill, 2005). Such conditions are often spatially and temporally variable. Fig. 3 provides a conceptual model that summarizes the potential for denitrification within an idealized subsurface environment. The conceptualization is based upon the many cited papers on denitrification and is intended to provide context for our review.

3. Electron donors used in denitrification

3.1. Organic carbon (heterotrophic denitrification)

Electrons needed for denitrification can originate from the microbial oxidation of organic carbon. Lack of organic carbon to provide energy to heterotrophic micro-organisms (denitrifying bacteria that use organic carbon as the electron donor) is usually identified as the major factor limiting denitrification rates in aquifers (Smith et al., 1988; Starr and Gillham, 1993; DeSimone and Howes, 1998; Jacinthe et al., 1998; Devito et al., 2000; Pabich et al., 2001).

Many factors affect the complex reactivity of 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). The rate of denitrification is most often related to the amount of dissolved organic carbon (DOC) in porewater or groundwater, or the amount of soluble organic carbon rather than the total amount of solid fraction organic carbon (f_{oc}) present on the geological strata. Burford and Bremner (1975) correlated the denitrification capacity of soils with the amount of water-soluble carbon and mineralizable (bioavailable) carbon. Cannavo et al. (2004) also related denitrification activity to DOC concentration. DOC levels in most aquifers are relatively low, typically <5 mg/l DOC (Rivett et al., 2007). Based on the example stoichiometry for the denitrification process relating nitrate and organic matter reaction given by Jørgensen et al. (2004):

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

Such concentrations of DOC can react only with relatively small quantities of dissolved oxygen or nitrate. The actual quantity and bioavailability of DOC hence become critical for the occurrence of denitrification in subsurface systems.

The above stoichiometry indicates that 1 mg carbon (C)/l of DOC is capable of converting 0.93 mg-N/l of nitrate to nitrogen
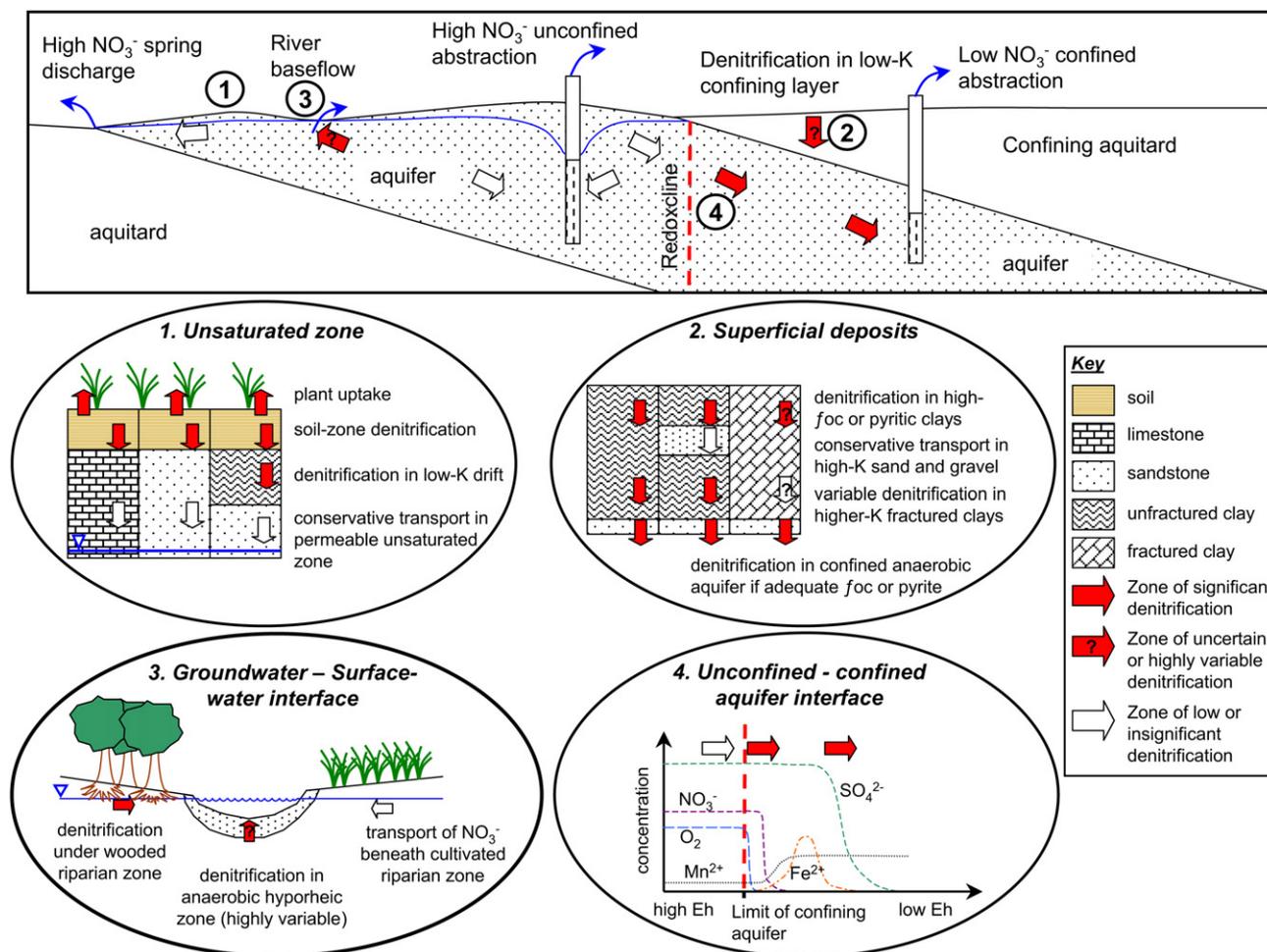
Fig. 3 – Conceptual model of denitrification occurrence in the subsurface environment.

gas. The DOC is, however, first oxidized by dissolved oxygen: this requires 1 mg-C/l DOC to convert 2.7 mg-O_2/l. In an air-saturated groundwater (ca. 10.3 mg-O_2/l at 12 °C), up to about 3.8 mg-C/l must therefore be oxidized before denitrification can commence (assuming complete coupling and with no account taken of bacterial death or C and N release back into the system). For an initially fully oxygenated groundwater (perhaps, for example, recently infiltrated recharge water), this could be an indicative concentration of DOC below which anaerobic conditions may not develop and denitrification hence would probably not occur. In practice, the actual availability of DOC in hydrogeological environments will vary. It is controlled primarily by the nature and quantity of the carbon source, but also by mineralization (microbial oxidation to its simplest forms, i.e. H_2O and CO_2), sorption and DOC attenuation (Jacinthe et al., 2003).

Siemens et al. (2003) 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 bioavailable. They concluded that denitrification in the groundwater below was being controlled by the limited 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. Beauchamp et al. (1989) indicated that denitrification in the presence of more complex organic molecules (e.g. proteins, lipids and lignin) was sometimes facilitated by bacteria performing fermentation – a redox process whereby organic compounds are both the electron donor and acceptor (Fig. 4). This tends to lead to changes in the organic chemistry of the solution as the bacteria break down complex molecules (e.g. sugars to alcohols). However, these reactions do not necessarily change the redox chemistry of the solution.

Kaiser et al. (2002) separated DOC into two categories: low-molecular-weight compounds (such as acetate) and high-molecular-weight compounds. The former were assumed to be more biologically reactive. Baker and Vervier (2004) confirmed that the rate of denitrification in an alluvial aquifer was best predicted by the concentration of low-molecular-weight organic acids. Corre et al. (1999) also described how water-extractable organic carbon (WEOC) from soils could be reasonably correlated with denitrification activity.

Solid-phase organic carbon contents of soil or geological deposits (typically expressed as the solid organic matter (SOM) or fraction of organic carbon (f_{oc})) may also give some
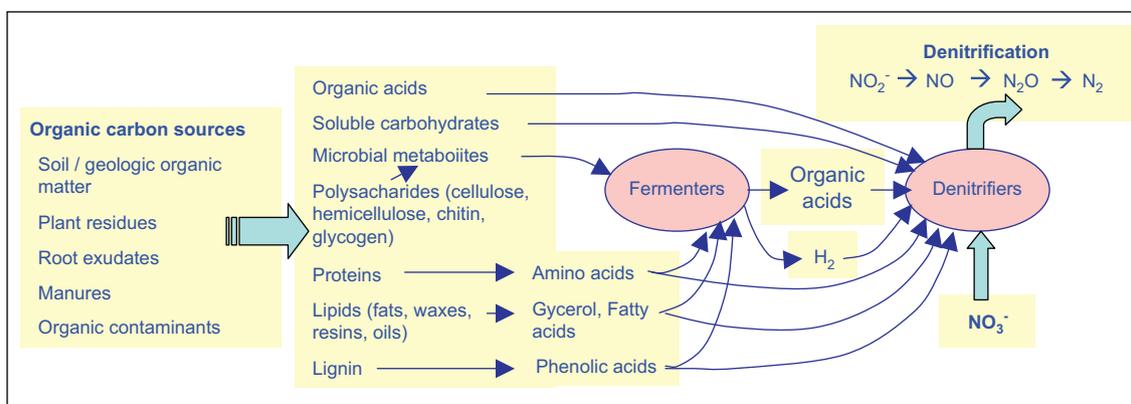
Fig. 4 – Schematic pathways of organic carbon transfer to fermenters and denitrifiers under anaerobic conditions (adapted from Beauchamp et al., 1989).

indication of the potential for denitrification. For example, Brettar et al. (2002) observed a positive correlation between denitrification rate and total organic carbon in a soil that contained immature carbon and which was assumed to have been relatively bioavailable. Dahl et al. (2007) report that an f_{oc} of 3% in riparian zone sediments was an effective indicator of the potential for denitrification. This would contrast with most aquifer environments where f_{oc} values are low (rarely exceeding 2% and more commonly around 0.1% or lower; Steventon-Barnes, 2002) and the carbon material is very mature, and hence generally assumed to be not that bioavailable. Recent research to characterize the composition of f_{oc} and its reactivity indicated that sediments containing more oxidized SOM (f_{oc}) are less reactive (via microbial action) to dissolved oxygen (Hartog et al., 2004). The geological history of the sediments could be correlated with reduction potential and sediments that had been exposed to aerobic conditions during deposition and diagenesis yielded SOM with a lower reactivity (Allen-King et al., 2002). Postma et al. (1991) and Kölle et al. (1983) both noted that denitrification was minimal when organic carbon was present as lignite or coal fragments.

Subsurface environments with high concentrations of labile organic matter and reducing conditions are likely to be particularly significant zones for denitrification. These include riparian zones (Haycock et al., 1993; Burt et al., 1999; Puckett, 2004; Puckett and Hughes, 2005; Mayer et al., 2006; Domagalski et al., 2008), hyporheic zones (Triska et al., 1989; Fischer et al., 2005; Pretty et al., 2006; Smith and Lerner, 2008) and aquifers affected by infiltration of DOC-rich surface water (Roberts and McArthur, 1998).

3.2. Organic contaminant carbon sources

In addition to the consumption of natural organic carbon during denitrification, the denitrifying bacteria may contribute to attenuation of organic pollutants in groundwater arising from contaminant sources. For example, benzene, toluene, ethylbenzene and xylene (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), partly due to

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.

Phenols, cresols and related compounds may also be degraded by denitrifying bacteria (Broholm and Arvin, 2000) though, at elevated contaminant concentrations, degradation is potentially inhibited (Spence et al., 2001). Chlorinated solvents such as tetrachloromethane (carbon tetrachloride) are subject to biodegradation under denitrifying conditions and nitrate addition has likewise been used in their bioremediation (Dybas et al., 1998). Lower-chlorinated solvents and related compounds such as dichloroethene and vinyl chloride may also biodegrade under denitrifying conditions (Rijnaarts et al., 1997). Denitrification does not, however, permit reductive degradation of most of the common, more highly chlorinated solvents such as trichloroethene (TCE) and tetrachloroethene (PCE), which require more strongly reducing conditions for dechlorination.
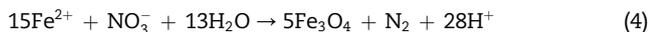
With sewage effluents, several studies (Robertson et al., 1991; Wilhelm et al., 1994; MacQuarrie et al., 2001) confirm that, although raw sewage contains significant labile organic carbon, much is oxidized coincidentally with, or prior to, ammonium oxidation in the unsaturated zone. Nitrate-rich, well-oxidized effluent may therefore contain only small amounts of carbon that might act as an electron donor. This is also likely to be the least bioavailable fraction of the organic carbon load. Any denitrification is largely due to the presence of other *in situ* electron donors. For example, DeSimone and Howes (1998) found that organic compounds in a wastewater plume were completely mineralized before denitrification started and the *in situ* electron donors in the sandy host aquifer contributed only a 2% decrease in the nitrogen load. In contrast, Spalding et al. (1993) studied a plume arising from sewage sludge disposal that contained sufficient bioavailable organic carbon to support denitrification. Singleton et al. (2007) and Gooddy et al. (2002) have both observed denitrification in strata beneath dairy operations that may centre on unlined cattle slurry lagoons.

In more complex landfill leachate environments, there is usually sufficient organic carbon to drive the redox condition

to methanogenesis (Christensen et al., 2000) with plume N typically present as ammonium. Nitrate is, therefore, generally not present in the plume where anaerobic conditions exist. However, reduction (denitrification) may occur at the outer plume halo where ambient groundwater nitrate mixes with the organic carbon and electron donors in the plume.

3.3. Reduced iron (autotrophic denitrification)

There is some evidence that groundwaters containing Fe^{2+} normally contain little or no nitrate (Korom, 1992). Reduction of nitrate by Fe^{2+} can be either abiotic, biotic, or both. The abiotic reduction process is not well understood. Davidson et al. (2003) demonstrated that Fe^{2+} acts to promote abiotic denitrification, in which Fe^{2+} reduces nitrate to nitrite, and is then regenerated by the oxidation of organic carbon. Alternatively, Fe^{3+} can precipitate as an oxyhydroxide or oxide mineral. Nitrite can then be abiotically reduced in organic-poor environments to gaseous nitrogen compounds by the further oxidation of iron (Korom, 1992). Examples of stoichiometric equations for these reactions are given below (in which the stable endpoint is nitrogen gas), but less complete reactions may have endpoints anywhere along the reduction pathway (Ottley et al., 1997):

$$10Fe^{2+} + 2NO_3^- + 14H_2O \rightarrow 10FeOOH + N_2 + 18H^+ \quad (3)$$

$$15Fe^{2+} + NO_3^- + 13H_2O \rightarrow 5Fe_3O_4 + N_2 + 28H^+ \quad (4)$$

Cu(II) appears to play a catalytic role in the abiotic reduction, with reaction rates significant at low μg/l quantities (Ottley et al., 1997). Ag(I), Cd(II), Ni(II), Hg(II) and Pb(II) may also catalyse the reduction reaction, with a slight effect noted in the presence of Mn(II). The direct role played by protons in the reactions means the reaction rate is pH-dependent, with the rate increasing with increasing pH (Ottley et al., 1997). Under neutral or alkaline conditions, 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.

The biotic process of nitrate reduction by Fe^{2+} can be due to the common bacterium *Gallionella ferruginea*. *G. ferruginea* is known to reduce nitrate to nitrite autotrophically in reduced iron environments (Korom, 1992); the nitrite produced can then be reduced abiotically. However, it 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. 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 deposited under anaerobic conditions, or strata subject to post-depositional mineralisation.

3.4. Reduced sulphur (autotrophic denitrification)

Electrons required 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 sulphite (SO_3^{2-}). Although in a groundwater treatment context elemental S(0) has been considered as an electron donor alternative to overcome biofouling concerns (Sierra-Alvarez et al., 2007), under typical aquifer conditions, iron (and sometimes manganese) sulphide (pyrite) is typically expected to be the electron donor (Korom, 1992):

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

This reaction is mediated by a wide range of autotrophs and heterotrophs including *Thiobacillus denitrificans*, which is recognized as the archetypal organism. Oxidation of sulphur, therefore, provides a viable alternative electron donor in carbon-limited systems (Kölle et al., 1985; Robertson et al., 1996; Kelly, 1997; Moncaster et al., 2000; Tesoriero et al., 2000; Broers, 2004). The susceptibility of pyrite to oxidation depends on its microscopic structure; hence not all pyrite in a sediment may be available for reaction (Kölle et al., 1985).

Although leading to decreased nitrate loading, autotrophic denitrification by sulphide may be detrimental to well field operations as sulphate concentrations, water hardness and borehole infrastructure corrosion potential may all increase (Kölle et al., 1985; van Beek and van Puffelen, 1987). Furthermore, ferric iron clogging may occur around the well screen due to oxidation and precipitation of iron and manganese that precipitate out of solution when aerobic conditions in the borehole are encountered. Release of heavy metals as a by-product of pyrite oxidation is well-known in the context of mine-water chemistry (Bowell, 2002), although this appears not to have been studied in the context of denitrification for which the effect may be partly mitigated by associated pH increases. A sulphate-reducing zone often lies downgradient from the nitrate-reducing zone in many anaerobic aquifers (Christensen et al., 2000). Thus, sulphate arising from denitrification may be transformed back to sulphide if organic carbon is available as an electron donor in the sulphate-reducing zone. Korom (1992) described a site where the denitrification reaction had a first-order half-life of 1–2 years, while the sulphate reduction reaction downgradient had a half-life of 76–100 years, causing a distinct sulphate plume to develop.

3.5. Other electron donors

Biodegradation of ammonium/ammonia may take place with or without nitrate formation. Certain autotrophic nitrifying bacteria oxidise ammonia (i.e. the electron donor) to nitrite, which is in turn reduced through denitrification to nitric oxide, nitrous oxide and molecular nitrogen (Wrage et al., 2001). A similar process can also be performed by bacteria operating the anaerobic ammonium oxidation (anammox) pathway (Strous and Jetten, 2004).

Denitrification reactions at some sites may be driven by multiple electron donors, for example, where organic carbon, sulphide and iron minerals are all available. In such cases, a multiple electron donor system may develop. At its simplest, this may occur from a change in lithology along a flow line; for example, Bölke et al. (2002) identified three different environments of denitrification within a superficial sand aquifer.

The recharge zone of the sandy aquifer contained both iron sulphides and organic carbon, but only the iron sulphide was being oxidized. At the discharge zone to a riparian wetland, iron sulphide acted as the electron donor at depth, but organic carbon was reduced in shallow horizons where it was more abundant. However, Aravena and Robertson (1998) identified a plume in which there was significant oxidation by organic carbon in a similar region of the aquifer although it seemed that sulphide was the primary electron donor. Postma et al. (1991) also identified a sand-and-gravel aquifer containing both organic carbon and pyrite, which both contributed to denitrification; reduction by pyrite was nevertheless the dominant denitrification process as the organic carbon appeared to be poorly bioavailable. Since the sulphide-oxidizing denitrification releases Fe^{2+}, reduced iron may also contribute to the denitrification potential (Kölle et al., 1985).

4. Denitrification activity: effects of environmental conditions

4.1. Nitrate concentration

Some workers (e.g. Morris et al., 1988; Smith et al., 1988; Korom et al., 2005) have reported that the kinetics of denitrification at concentrations >1 mg-N/l are zero order (i.e. independent of concentration), suggesting that supply of electron donors controls the rate. Excess nitrate concentrations affect the denitrification process by inhibiting the formation of N_2 gas and causing the denitrification process to terminate with the formation of N_2O (Blackmer and Bremner, 1978). These concentrations appear case specific but, in some cases, even low concentrations affect the ratio of $N_2O{:}N_2$ evolved. Magalhàes et al. (2003), for example, showed an increase in the $N_2O{:}N_2$ ratio from 0.11 to 0.34 associated with an addition of 0–4 mg-N/l, 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 (see below).

4.2. Oxygen concentration

The denitrification process is thermodynamically less favourable than the reduction of dissolved oxygen (Fig. 1). In a system that contains oxygen, nitrate and organic carbon, the oxygen will normally be the preferred electron acceptor meaning that denitrification can be considered as a predominantly anaerobic process. Numerous field studies illustrate how significant denitrification takes place only once the dissolved oxygen concentration falls below a certain low threshold (Table 1). There is little consensus but it seems reasonable to assume that, given all other prerequisites, denitrification will probably occur at dissolved oxygen concentrations below 1 mg-O_2/l and perhaps below 2 mg-O_2/l. In cases where they have been quantified (e.g. DeSimone and Howes, 1998), denitrification rates tended to be greater in regions of lowest oxygen concentration.

However, micro-organisms in sediments do not necessarily 'experience' the same concentrations as those measured 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} ml. Therefore, only a very small volume of water, relatively isolated from mixing with the bulk oxygenated groundwater, is needed within which denitrifying bacteria can begin to respire nitrate. The threshold concentrations in Table 1 are thus a guide to the conditions under which denitrification can occur.

Carter et al. (1995) isolated several species of bacteria from soils that were capable of respiring oxygen and nitrate simultaneously under aerobic conditions (at up to 80% air saturation). Aerobic denitrifying fungi also occur (Cannavo et al., 2004). However, reported examples of aerobic denitrification in the groundwater environment are few. In studies in which aerobic denitrification has been postulated, denitrification actually seems more likely under locally anaerobic conditions within micro-sites in particulate organic matter (Hammersley and Howes, 2002), heterogeneous organic-rich patches of sediments (Jacinthe et al., 1998) or biofilms (Seiler and Vomberg,

Table 1 – Approximate limit of dissolved oxygen concentration in groundwater below which denitrification has been observed in the field		
Dissolved oxygen concentration (mg/l-O_2)	Conditions[a]	Reference
4	Agricultural fertilizer plume	Bölke and Denver (1995)
2–3	Agricultural fertilizer plume	Tang and Sakura (2005)
2	Literature survey – varied conditions	Bates and Spalding (1998)
2	Septic waste plume	Gillham (1991)
1.2	Agricultural fertilizer plume	Gallardo and Tase (2005)
1	Agricultural fertilizer plume	Puckett and Cowdery (2002)
1	Agricultural fertilizer plume	Bölke et al. (2002)
1	Landfill plumes	Christensen et al. (2000)
1	Natural (arid zone, $T \approx 30\,°C$)	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)
a Groundwater temperatures are seldom presented.		

2005). The presence of such micro-anaerobic environments could explain why there are some unexpectedly high dissolved oxygen concentrations in the bulk sample results in Table 1.

4.3.   Nutrient and micro-nutrient availability

Denitrifying bacteria obtain energy for metabolism and growth from the oxidation of organic carbon, sulphide minerals or reduced iron and manganese. Their metabolic requirements for nitrogen can be met by available NH_4^+ or organic N in the environment, or from the direct assimilation of nitrate. They also require carbon, phosphorus, sulphur and micro-nutrients (such as B, Cu, Fe, Mn, Mo, Zn and Co) for effective metabolism. Although most groundwaters contain adequate concentrations of the necessary minerals to support microbial growth (Champ et al., 1979), oligotrophic systems where nutrients or micro-nutrients are absent or present only in small quantities may limit the extent of bacterial growth and hence denitrification.

Phosphorus availability might be expected to be a key limiting factor in aquifer systems due to its often reduced mobility relative to nitrate. 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 only to nitrite). It was found that 0.16 mg-P/l was required to effectively remove 17 mg-N/l nitrate without significant accumulation of nitrite (a molar ratio of 235:1 N:P). Predominant sorption control on P mobility in aquifers (and hence N:P ratios) has been demonstrated in reactive transport modelling of column experiments (Stollenwerk, 1996; Isenbeck-Schröter et al., 1993) and field-observed septic-system wastewater plumes (Spiteria et al., 2007). Sorption is mostly to Fe oxides (Robertson, 1995) and calcium carbonate (Corbett et al., 2002) with the former typically exhibiting sorption coefficients three orders of magnitude greater (Krom and Berner, 1980). In coastal submarine groundwater discharges, P may hence be strongly attenuated relative to N in zones of iron oxide accumulation (iron curtains) at the freshwater–saltwater interface potentially leading to changes in eutrophication occurrence (Charette and Sholkovitz, 2002). Field-observed retardation factors for P of 20–100 are reported by Robertson et al. (1998) for septic-system plumes in nine sandy aquifers in North America and one sandy till, while nitrate underwent no retardation. Spiteria et al.'s (2007) modelling of some of those plumes indicates P sorption/desorption may lead to complex spatial and temporal distributions of N:P ratios developing as the source term varies with significant contrasts apparent in calcareous and non-calcareous Fe oxide-rich systems. Such septic-system studies still, however, indicated that denitrification was limited by DOC availability (Wilhelm et al., 1994; MacQuarrie et al., 2001; Spiteria et al., 2007). Hence although it is clear that N:P ratios may vary significantly in groundwater systems, there is a lack of evidence of in-aquifer denitrification being limited by P availability.

The presence of sulphur as sulphate and thiosulphate has 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) cite examples where the presence of sulphide alleviates the acetylene blockage of the conversion from N_2O to N_2.

4.4.   pH

The pH range preferred by heterotrophic denitrifiers is generally between 5.5 and 8.0 (Rust et al., 2000). pH values outside this range may hinder the denitrification process, but the optimal pH is site-specific because of the effects of acclimation and adaptation on the microbial ecosystem. In addition, concentrations of dissolved phosphate 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 pH controlled.

Strongly acidic environments (pH < 5) inhibit denitrification and tend to arrest the denitrification chain with the formation of nitrite or N_2O (Brady and Weil, 2002). This may occur where organic wastes are oxidized to organic acids and the aquifer is not well-buffered (Wilhelm et al., 1996; DeSimone and Howes, 1998). In well-buffered calcareous aquifers, such acidification is unlikely (Amirbahman et al., 1998; Robertson et al., 1998). Abiotic denitrification has been observed in very low pH (<4.5) soils (Beauchamp et al., 1989). Denitrification itself can increase pH by releasing CO_2 and hydroxide (OH^-). Normally these combine to yield HCO_3^-, but if the production of OH^- exceeds that of CO_2, 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.   Temperature

The optimum temperature for denitrification is between 25 and 35 °C, but denitrification processes will normally occur in the range 2–50 °C (Brady and Weil, 2002) and possibly beyond, where bacteria have evolved to cope with specific environmental conditions. Groundwater temperatures are typically around 10 °C (in northern Europe), with the exception of shallow groundwaters impacted by extreme surface temperatures. Reaction rates are typically assumed to double for every 10 °C increase in temperature (i.e. Arrhenius rate law). Lind (1983) confirmed that denitrification in subsoil at *in situ* (10 °C) temperatures was significantly slower than at laboratory temperatures (25 °C). However, the observed increase in rate from 10 to 25 °C varied considerably for different soils (by factors ranging from 1.7 to 23), suggesting that additional factors may also be involved.

It is difficult to observe temperature dependency of denitrification rates in the relatively stable temperatures of the groundwater environment. Nevertheless, Saunders and Kalff (2001) and Grischek et al. (1998) observed this dependency in the bed sediments of a lake and a river, respectively. Saunders and Kalff (2001) observed that a 5 °C increase resulted in a 10-fold increase in denitrification rate.

Robertson et al. (2000) demonstrated a correlation between water temperature and denitrification rates in a permeable reactive barrier system. Denitrification was observed down to 2 °C; between 2 and 5 °C, rates were approximately 5 mg-N/l/day and; between 10 and 20 °C, rates increased to 15–30 mg-N/l/

day. Christiansen and Cho (1983) reported 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 ascribed this to aerobic denitrifying fungi that 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 which denitrification can become established.

4.6. Salinity

High salinity (such as in wastewaters) is known to inhibit, but not necessarily completely arrest, denitrification. Relatively few studies are available; nevertheless, Dinçer and Kargi (1999) showed that denitrification was inhibited by concentrations of salt greater than 20 g/l sodium chloride (57% sea water), while Ucisik and Henze (2004) found that denitrification rates were reduced to 10% of the maximum when chloride concentrations were between 5 and 97 g/l chloride. In estuarine and marine environments, however, denitrification rates do not appear to be affected by the salinity in which they occur (Kana et al., 1998; Magalhàes et al., 2003).

4.7. Inhibitory substances

Denitrification can be inhibited by the presence of heavy metals, pesticides and pesticide derivatives (Bollag and Henneringer, 1976; Bollag and Barabasz, 1979; Bollag and Kurek, 1980; Hunter, 2003), and by the presence of other organic compounds at such elevated concentrations that they are toxic to denitrifying bacteria (e.g. Spence et al., 2001). For example, Bollag and Henneringer (1976) investigated the effect of Cd, Cu, Pb, and Zn on denitrifying activity. Their observed relative order of toxicity (i.e. inhibition of denitrification activity) was similar to that observed by Bååth (1989) of $Cd > Cu > Zn > Pb$ in their wider ranging review of metal toxicity influences on soil micro-organisms and microbially mediated soil processes (largely of temperate forest soils). Modelling of relationships between denitrification activity and Cd in a wastewater treatment setting indicated that concentrations resulting in 50% inhibition would be around 12 mg/l Cd for such test conditions (Gumaelius et al., 1996). Such concentrations would be encountered only within an extremely polluted groundwater.

The effects of pesticides on denitrifying activity have been assessed by Cervelli and Rolston (1983), Yeomans and Bremner (1985), Martinez-Toledo et al. (1996) and Sáez et al. (2003). The laboratory study by Sáez et al. (2003) examined eight common pesticides (aldrin, lindane, dimetoate, methylparathion, methidation, atrazine, simazine and captan), measuring denitrifying activity via N_2O release and nitrite accumulation. Captan, a fungicide, totally inhibited growth and activity of the tested strain of *Paracoccus denitrificans*; the other pesticides led to a growth delay only at an early time (<4 days). The denitrifying activity of *P. denitrificans* was negatively affected by all the pesticides with inhibition of N_2O release and accumulation of nitrite.

However, Sims (1990) reported a number of instances where pesticides had no effect on, or even stimulated, denitrification. Subsequently, Jørgensen et al. (2004) found that denitrification in a column of till depleted in natural DOC was stimulated by the addition of a pulse of pesticides (bentazon, MCPA, MCPP, fenoprop and propoinol), which acted as a source of organic carbon. Co-application of a wide range of pesticides with nitrate in many agricultural settings makes such an observation significant when determining the fate of both pesticides and nitrate. Studies on the natural attenuation of MCPP (mecoprop) have reported inconsistent biodegradation under denitrifying conditions (Buss et al., 2006). Further research is required to address whether such anthropogenic sources provide a DOC source and/or pose toxicity concerns at elevated concentrations.

4.8. Sediment pore size

Intergranular aquifers provide a high surface area to volume ratio for microbial growth and the pore spaces represent the regions of greatest biomass and metabolic activity (Blakey and Towler, 1988). The exception is when pore spaces are too small to permit microbial growth. This may occur in fine-grained aquitards, but also within the matrix of some dual porosity aquifers – notably some Jurassic and Carboniferous Limestones and Cretaceous Chalk deposits that form major aquifers in many parts of Europe. A large microbial population may not develop in the matrix pore space due to the small pore sizes (West and Chilton, 1997). It was concluded from examination of the Cretaceous Chalk that movement of bacteria (typical diameter of 1 µm) was precluded by the small pore sizes of the Chalk where median pore-throat diameters ranged from 0.2 to 0.7 µm (Whitelaw and Rees, 1980; Rees, 1981). Biotic (denitrification) activity appeared to be restricted to the fissure wall vicinity (Whitelaw and Edwards, 1980; Foster et al., 1985; Johnson et al., 1998). These observations contrast with those of Seiler and Vomberg (2005) who found, in a karstic reef limestone in the Jura of Southern Germany, that the pore size (about 50 µm) was sufficient for biofilms to form and high flow velocities within the fractures actually tended to inhibit biofilm growth due to shear stresses.

4.9. Microbial acclimation

Acclimation is 'lead time' before a microbial population can adapt to new conditions such as the presence of a new nutrient source or other changed environmental conditions. Denitrifying bacteria appear ubiquitous in the natural environment and can respond quickly to nitrate inputs provided other environmental conditions are conducive to their activity.

In some cases, denitrifying populations appear to maintain their denitrifying enzymes when conditions are unsuitable for nitrate reduction (e.g. Smith and Tiedje, 1979). In such cases, the population is able to undertake denitrification rapidly once oxygen depletion occurs and any observed acclimation period will reflect the time taken to increase the denitrifying population.

In other cases, it appears that populations may synthesise the enzymes involved in denitrification in response to nitrate supply. 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 only from infrequent storm pulses. Denitrification rates were higher at the upgradient edge of the site where nitrate exposure was more frequent. It was concluded that a population of denitrifying bacteria was maintained between inflow pulses, but that denitrifying enzyme activity appeared to improve with increased exposure to nitrate and required a 'priming' period of several hours for enzymes to be re-established on exposure. Cannavo et al. (2004) found a similar pattern of denitrifying potential 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 (<1 m), at greater depths the limiting factor was exposure to nitrate.

5. Nitrate depletion mechanisms other than denitrification

5.1. 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). This was represented by Robertson et al. (1996) as

$$2H^+ + NO_3^- + 2CH_2O \rightarrow NH_4^+ + 2CO_2 + H_2O \qquad (6)$$
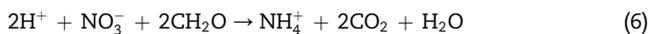
The DNRA reaction occurs under much the same conditions as denitrification but is less commonly observed in practice. The partitioning of nitrate between denitrification and DNRA is believed to be controlled by the availability of organic matter: DNRA is the favoured process when nitrate (electron acceptor) 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 which carry out DNRA are obligate anaerobes (Hill, 1996) and 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 oxidized back to nitrate or taken up by vegetation. However, the sorption and ion exchange of ammonium (Buss et al., 2004) and nitrite (Davidson et al., 2003) are expected to be significant in many aquifer systems, so DNRA may provide a mechanism for the temporary attenuation of nitrate.

DNRA is rarely found to be the dominant nitrate reduction mechanism in groundwater systems, although Bulger et al. (1989) observed DNRA of nitrate in groundwater flowing beneath waste stabilization ponds discharging organic-rich wastewater. Smith et al. (1991) suggested 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 oxidized. Ammonium taken from within the nitrate plume had significantly enriched δ^{15}N isotope composition compared with ammonium from the source or in the main plume. Nitrate was also significantly enriched in δ^{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. They found that glycine (an amino acid) and glucose stimulated some formation of ammonium though, for glycine, this may have been partly through mineralization of the amino group, while most nitrate was denitrified or converted to biomass. Acetate and formate (both fermentation products of organic wastes) only caused decreased 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 indicate that DNRA is the dominant nitrate reduction process in a system.

5.2. 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 (Hill, 1996). In some environments, however, there is evidence that conversion to biomass growth can become an important mechanism for nitrogen removal, or at least, short-term retention. For example, Kelso et al. (1999) showed that in the presence of some organic substrates, up to 50% of nitrogen depleted from groundwater could be converted to biomass.

Apart from systems where microbial biomass development is extensive (e.g. following a release of readily biodegradable 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} bacterial cells (Holm et al., 1992). Assuming that the dry weight of a 'typical' bacterial cell is 1 pg, this corresponds to a total biomass of 0.025 g/kg. If the porosity of the sediment is 30% and its bulk density is 1600 kg/m^3, this would equate to a microbial load of only 4 mg/l as N. Furthermore, rapid bacteria die-off may often be anticipated and lead to N release as ammonium back into groundwater.

5.3. Nitrate removal via phreatophytes

Although most plants and vegetation acquire their nitrogen from the soil zone, some trees have deep-root systems that extend to the water table (phreatophytes). These include poplars, willow and cottonwood that are able to withdraw water from the unsaturated zone and shallow groundwater, and have been increasingly used to remediate groundwater contaminant plumes (Schnoor et al., 1995; Hirsh et al., 2003). Phreatophytes may interact with nitrate locally present in groundwater and cause removal via uptake processes or denitrification enhancement by plant root exudates. Indeed, Jordahl et al. (1997) confirmed denitrifiers were four times higher in the rhizosphere of poplars compared with surrounding soils. Haycock and Pinay (1993) and Clément et al.

(2002) measured differences in the denitrification capacity of riparian zones with different vegetation. Paterson and Schnoor (1993) observed the vegetative alteration of nitrate fate in the unsaturated zone, and Licht and Schnoor (1993) illustrated the use of poplars in Iowa to control groundwater nitrate concentrations in a riparian buffer zone. In the latter, nitrate in groundwater fell from 34 mg-N/l at the edge of a corn field to 1.8 mg-N/l below a downgradient poplar buffer strip, and then to 0.7 mg-N/l downgradient at the edge of a watercourse. Similar examples are provided in the review by Mayer et al. (2006) that illustrated nitrogen removal effectiveness varied widely among riparian zones studied. Such riparian zone studies generally recognize the critical role of phreatophytes, but without quantitatively assessing phreatophytic contributions relative to other nitrate attenuation processes occurring.

6.   Conclusions and recommendations

Elevated nitrate concentrations in groundwater may lead to the derogation of precious aquifer resources and the eutrophication of surface waters. Within Europe, these risks are managed through Member States' implementations of the Nitrates and Water Framework Directives. Understanding of processes controlling the natural attenuation of nitrate, which may lead to risk reduction, is critical to the implementation of these directives.

We have reviewed current understanding of denitrification in the subsurface groundwater environment. Conditions under which nitrate attenuation, in particular denitrification, might be predicted to occur are reasonably well-known. Denitrification requires all the following conditions to be met:

- presence of nitrate, denitrifying bacteria and electron donor (organic carbon, reduced iron and/or reduced sulphur);
- anaerobic conditions (dissolved oxygen concentrations less than around 1–2 mg/l);
- favourable environmental conditions (e.g. temperature, pH, other nutrients and trace elements).

Because denitrifying bacteria appear to be almost ubiquitous in the subsurface, the critical limiting factors for denitrification are the presence of anaerobic conditions and the presence of a suitable electron donor (most commonly organic carbon). The availability of the latter is usually identified as the major factor limiting denitrification rates in aquifers. The effects of other ambient environmental conditions such as nitrate concentration, nutrient availability, pH, toxins and microbial acclimation do not appear to be as significant, with secondary influences likely to be apparent on denitrification rates in most circumstances.

Although the biogeochemical processes controlling nitrate attenuation are reasonably understood, further research is still warranted. As summarized in Fig. 2 and demonstrated elsewhere (Rivett et al., 2007), conditions conducive to denitrification may not be encountered in many unconfined aquifers. Lengthy travel times, however, mean that improved understanding of even low rates is required as these may still partially alleviate nitrate problems.

Denitrification is more probable in confined aquifer or near-river environments, i.e. the riparian and hyporheic zones (Fig. 2). Future reliance on these settings may increase due to potential transfers of abstraction supplies to confined aquifers as pressures on unconfined resources increase, and the capacity of riparian and hyporheic zones to limit eutrophication declines. Within these contexts, we recommend that the following research is considered to improve understanding:

1. *Organic carbon electron donors*: composition and bioavailability to denitrifying bacteria, influence of pH, temperature, oxidants and occurrence as colloids or nano-particles.
2. *Sulphur and iron electron donors*: biotic and abiotic autotrophic denitrification reaction nature, individually and within multiple electron donor systems.
3. *Physical environment*: restrictions on microbial activity due to matrix pore size exclusion and shearing forces operating in high flow fractures.
4. *Environmental conditions*: pH influences in poorly buffered environments; salinity influences in coastal or salinized soil settings; inhibitions by metals or lack of nutrients, especially phosphorus.
5. *Attached versus free-phase denitrifying bacteria*: relatively little is currently known about the relative contributions of attached (biofilm) and planktonic bacteria to denitrification in aquifers.
6. *Co-contaminant influences*: contrasting inhibitory, toxic, electron donor influences of pesticide concentration and type, and the toxicity of common organic pollutants to denitrifying bacteria.
7. *Quantification of conditioned denitrification rates*: quantified rate data conditioned to the biogeochemical environment that may be scaled from laboratory to field.
8. *Quantification of field-scale denitrification*: field assessment of denitrification occurrence and rates is challenging; improved field methods are required.

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