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DENITRIFICATION IN SUBSOILS AND GROUNDWATER IN IRELAND

by

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Thesis submitted to The University of Dublin, Trinity College for the Degree of Doctor of Philosophy (PhD)

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DECLARATION

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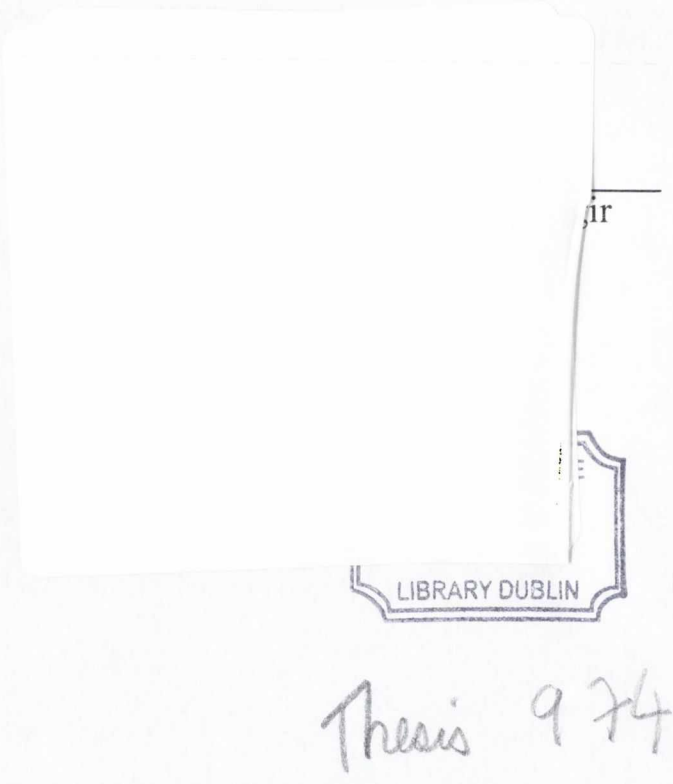

ABSTRACT

Excessive reactive nitrogen (N) in groundwater is of huge concern to surface water quality and atmospheric nitrous oxide (N_2O) emissions via denitrification. Understanding denitrification rates and factors controlling denitrification over space and time is crucial for quantifying the effects of human activity on the N cycle, and for managing and mitigating the severe environmental consequences associated with excessive reactive N. Despite the extensive research in the topsoil, denitrification in subsoils, below the rooting zone, is not well understood. Subsoil (only at Johnstown Castle, JC) and groundwater denitrification rates and ratios of N_2O/N_2O+N_2 were investigated at four agricultural sites: (JC) Johnstown Castle, grassland, (SH) Solohead, grassland, (OP) Oak Park, arable land and (DG) Dairy Gold, grassland. The impacts of site hydrology, hydrogeology, hydrochemistry and microbiology on denitrification rates were considered. Subsoil denitrification rates were investigated in intact soil cores collected from 0-0.10, 0.45-0.55 and 1.20-1.30 m depths. Soil cores were amended with 90 mg nitrate (NO_3^--N) kg^{-1} dry soil as KNO_3, and treatment solution consisted of (i) a control, (ii) 150 mg glucose-C, and (iii) 150 mg DOC (dissolved organic carbon) kg^{-1} dry soil. The added C sources in subsoils satisfactorily increased NO_3^- depletion via denitrification where the mole fraction of N_2O were further reduced to N_2 during diffusional transport through the soil profile to the atmosphere and/or to groundwater. Denitrification losses of the added N decreased significantly with soil depth and were increased by the addition of either C source. The ratios of N_2O to N_2O+N_2 differed significantly only between soil horizons, being higher in the A (0.58 – 0.75) than in the deeper horizons (0.10 – 0.36 in B and 0.06 – 0.24 in C), indicating the potential of subsoils for a more complete reduction of N_2O to N_2. Groundwater systems have the potential for the natural NO_3^- reduction but it shows a large variability between different agricultural sites due mainly to their complex hydrologic (e.g. K_{sat}, changes in groundwater table depth etc.) and hydrogeochemical (redox chemistry i.e. DO and Eh; DOC and other electron donors like reduced Fe and S, NO_3^- concentration, pH etc.) variabilities. In situ monitoring of N_2O and N_2 in groundwater (5-50 m depth) between Feb, 2009 and Jan, 2011 on a monthly basis revealed that denitrification is a significant pathway of NO_3^- reduction consuming 46-77% at JC and SH and 4-8% at OP and DG sites of delivered N and resulting in, respectively, 1-4 and 11-15 mg L^{-1} net NO_3^--N. Mean N_2O emission factors were higher than the new default IPCC values (2006) and more similar to older IPCC values (1997). Denitrification functional genes (nitrous oxide reductase '*nosZ*' and nitrite reductase '*nir*') were detected at all sites and depths with similar quantities, which therefore imply that groundwater denitrification is controlled mainly by hydrogeology and

geochemical conditions. Multiple electron donors (organic C and Fe/S minerals); low Eh (<100 mV), DO (<2 mg L^{-1}) and permeability (K_{sat}<0.005 m d^{-1}); and a shallow unsaturated zone (<2 m bgl) appeared to be highly favorable for denitrification. Although groundwater N_2O and N_2 can leach to groundwater table (GWT) with recharge, in situ ^{15}N tracer test, being measured at JC and OP sites, revealed that they were also produced in the shallow and deep groundwaters. In situ denitrification rates were equivalent to a weighted average of 3.92 and 0.09 mg NO_3^--N L^{-1} d^{-1}, respectively at JC and OP, which accounted for 24.5 and 0.33% of the injected N. The dissimilatory NO_3^- reduction to ammonium (DNRA) contributed, respectively, to 0.04 and 0.03 mg N L^{-1} d^{-1}, which accounted for 0.08 and 0.05% of injected NO_3^- at JC and OP. Mean total NO_3^- reduction via denitrification plus DNRA was 166.7 and 6.3 μg kg^{-1} d^{-1}, which were equivalent to 4.00 and 0.14 mg N L^{-1} d^{-1} that were accounted for 25 and 0.53% of the applied N. A ^{15}N tracer test in shallow groundwater showed that cover crop (mustard) after spring barley significantly reduced NO_3^- to N_2O to N_2. Groundwater can be an important source of biogenic CO_2 and CH_4 emissions to the atmosphere containing 35, 27, 11 and 33 mg C L^{-1} as CO_2 and 246, 31, 5 and 1 μg C L^{-1} as CH_4, respectively, at JC, SH, OP and DG. Annual losses of dissolved N from terrestrial ecosystems to surface waters via groundwater were 12, 8, 38 and 27% of N input, of which 60, 26, 85 and 90% was NO_3^--N. The results suggest that geologic-based identification of areas with high and low groundwater denitrification potential can be an important N management tool in agricultural systems. Emissions of dissolved C, N and greenhouse gases via groundwater is an important component of farm scale C and N balances and global C and N budgets.

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ABBREVIATIONS and UNITS

AET	Actual Evapotranspiration
AOD	Above Ordnance Datum
API	American Petroleum Institute
bgl	Below ground level
BBC	British Broadcasting Corporation
Br	Bromide
C	Carbon
d	Day
DAF	Department of Agriculture and Forestry
DC	Dissolved Carbon
DG	Dairy Gold
DEHLG	Department of Health, Environment and Local Government
DN	Dissolved Nitrogen
DoE	Department of Environment
Defra	Department for Environment, Food and Rural Affairs
DGA	Dissolved Gas Analyser
EEA	European Environment Agency
EC	European Community
EU	European Union
EPA	Environmental Protection Agency
EQS	Environmental Quality Standard
GSI	Geological Survey of Ireland
GPS	Global Positioning Systems
GWT	Groundwater Table
ha	Hectare
IGV	Interim Guideline Value
IPCC	Intergovernmental Panel on Climate Change
JC	Johnstown Castle
KBr	Potassium Bromide
MAC	Maximum Admissible Concentration
MAFF	Ministry of Agriculture Fisheries and Food
MoE	Ministry of Environment
N	Nitrogen
NBL	Natural Background Level
NET	Net Ecosystems Productivity
NRA	National Rivers Authority
OP	Oak Park
OECD	Organization for Economic Co-operation and Development
PET	Potential Evapotranspiration
SMD	Soil Moisture Deficit
SH	Solohead
TN	Total Nitrogen
TDN	Total Denitrification
UNEP	United Nations Environment Programme
USEPA	United States Environment Protection Agency
USGS	United States Geological Survey
WFD	Water Framework Directive
WFPS	Water Filled Pore Space
WSOC	Water Soluble Organic Carbon
y	Year

Metric units are used throughout the thesis

CHAPTER 1. INTRODUCTION

1.1 Overview of this chapter

This chapter represents the significance of the research in light with the water and air quality concerns and the major objectives of the research. An overview of the agricultural system in Ireland is given in section 1.3 and the status of NO_3^- in Irish ground and surface waters was stated in section 1.4. The serious health and ecological hazards of NO_3^- in the environment is highlighted in section 1.5. The need for this research in light with the legislative frame work in Europe is stated in this chapter.

1.2 General introduction

The impacts of intensification of agricultural production are a major threat to the ecology of agro-ecosystems (Stoate et al., 2009). Within the past few decades, humans have dramatically altered the earth's nitrogen (N) cycle (Groffman et al., 2009), which globally more than doubled the reactive N production (Sutton et al., 2009). Excessive reactive N represents a cascade of environmental problems (Zhu et al., 2011) that are only now beginning to be fully appreciated (Sutton et al., 2009). The N cascade is an increasingly important global issue with multiple impacts on terrestrial, aquatic and atmospheric environments (Galloway et al., 2008). The global food chain has a mean N use efficiency of 14% for plant products and 4% for animal products, and the remainder is dissipated into the environment (Sutton et al., 2009). In agricultural systems, N in excess of plant and animal needs can leach to groundwater and enter surface waters (Schipper et al., 2010). Nitrate contamination in groundwater and surface waters is a major factor affecting estuarine eutrophication (Hakason et al., 2007; Howarth and Marino, 2006) and drinking water supplies in many European countries (EEA, 2005). Safeguarding water quality is therefore now a top priority for European Environment Policy (EC Report, 2010). In addition, indirect nitrous oxide (N_2O) emission from groundwater is an important component of global N_2O budget, accounting for approximately 10% of the global emissions, but is poorly understood. Nonetheless, the global N_2O budget has high uncertainty of estimation (Weymann et al., 2011) due mainly to the lack of measurement data.

For the conservation of groundwater resources and sustainable development of agriculture and industry, it is crucial to evaluate the NO_3^- attenuation potential in groundwater. There are several pathways through which excess N is removed in soil and groundwater before reaching the surface waters: some of which are temporary (plant uptake, microbial assimilation, dissimilatory nitrate reduction to ammonium-DNRA) and some are permanent (denitrification and anammox). Denitrification, an important sink for N inputs (Alexander et al., 2009; Böhlke et al., 2009), converts NO_3^--N to $NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. The N_2O, intermediate product of denitrification, is a potent greenhouse gas (Prather et al., 2001), with a global warming potential 300 times of CO_2 (IPCC, 2007), whereas N_2 is highly inert without any harmful environmental consequence. However, much needs to be learned about the magnitude of the various biochemical pathways of N_2 production in the environment (Megonigal et al., 2004). Denitrification is a function of local environmental conditions and can reduce NO_3^- by up to 100% while it is passing through and from landscape to the receptors. However, the in situ controlling factors of denitrification are not well known (Böhlke et al., 2009), even though they are of keen interest (Alexander et al., 2009). In addition, simulation or modelling of NO_3^- transport, field scale N balance, as well as global N_2O and N_2 balances, will be seriously misleading without measuring denitrification extensively in the existing hydrogeological and mineralogical conditions, as its extent varies largely with local environmental conditions (Seitzinger et al., 2006). There is, therefore, still much to be learned about denitrification rates and controlling factors across the entire range of natural ecosystems (Megonigal et al., 2004; Seitzinger et al., 2006). Denitrification can simultaneously cause carbon dioxide (CO_2) and methane (CH_4) production due to consumption of C as energy source.

In Ireland, groundwater beneath some agricultural systems is contaminated with NO_3^- (<2 to >50 mg NO_3^- L^{-1}; McGarrigle et al., 2010). The OECD (2009) urged Ireland to strengthen measures to achieve "good ecological status'' for Irish waters by 2015, paying special attention to eutrophication. The requirement for "good ecological status'' for Irish waters is a requirement of the EU Water Framework Directive (WFD; EC, 2000) and the associated Irish Regulations enacted (European Communities Environmental Objectives (Surface Waters) Regulations 2009 (S.I. No. 272 of 2009) and European Communities Environmental Objectives (Ground Water) Regulations 2010 (S.I. No. 9 of 2010)). It also urged Ireland to take proper actions to reduce N_2O emissions to achieve the 13% reduction over 1990 levels by 2008-2012. The EU legislation on nitrates aims at reducing water pollution by NO_3^- from agricultural sources and at preventing further pollution. Our main

hypotheses in these connections are (i) several biochemical NO_3^- removal pathways are occurring in the Irish agroecosystems, which largely vary with the local hydrogeological conditions and land use, giving 'hot spots' and 'hot moments'; and (ii) NO_3^- removal can cause pollution swapping to atmospheric N_2O emissions, with concurrent emissions of CO_2 and CH_4.

1.3 Principal agricultural systems and N inputs in Ireland

Agriculture in Ireland is primarily a grass based industry. The land area of Ireland is 6.9 million hectares, of which about 4.2 million hectares are used for agriculture (about 64% of total land area) and 745,456 hectares for forestry (about 10.8% of total land) (Teagasc, 2010). Approximately 80% (3.36 million ha) of the Irish agricultural area is devoted to grass (silage, hay and pasture), 11% (0.46 million ha) is in rough grazing and the remainder 9% (0.38 million ha) is allocated to crop production (Teagasc, 2010). Beef and milk production currently account for close to 60% of agricultural output at producer prices. Food production for the growing population intensively involved high N inputs. Nitrogen inputs to land have been increasing all over the world, stemming from food and energy production activities supporting the growing populations (Galloway et al., 2004). Maximization of grazed grass in the diet of dairy cow is now Ireland's focus in efforts to increase milk production. This involves compact spring calving to grass over a 90-day period (February-April). Lactation length is 280-300 days where 90% of the annual diet is either grazed grass or grass-silage (Humphreys et al., 2003). In Europe, dairy cows excrete 80%, on average, of the N that they consume (Oenema, 2011). Intensive agriculture can contribute to water pollution when excess fertilizers, sewage, or slurry/animal excreta are washed off the land into the water or leached into the groundwater. From a survey in 21 dairy farms during 2003-2006, Treacy et al. (2008) projected that mean stocking density was 202 kg N ha^{-1} (N excreted by livestock) and mean fertilizer N application was 223 kg ha^{-1}. Fertilizer N application accounts for 80% of total N import in grazed grassland whereas concentrate feed was the second largest source of N (15% of total inputs). Treacy et al. (2008) estimated the mean total N import to grassland was 288 kg N ha^{-1} in 2006, where as in the same year the mean total N surplus was 232 kg N ha^{-1}. This intensive grass-based farming contributes to large inputs of fertilizer N to sustain high milk output per hectare (Treacy et al., 2008). This indicates the potential risk of nitrate delivery to groundwater and surface waters from the agricultural N inputs.

Agricultural wastes i.e. animal manures and dirty water/farm washout are of great environmental concern if they are not handled wisely, because all animal manures and dirty water contain significant amount of nutrients, mainly N and phosphorus (P) (Pain and Smith, 1993). These disposals are mainly spread on agricultural lands, but poor control of these disposals has contributed to substantial degradation of water quality (McGarrigle, 2002). In addition, some 55 ton ha^{-1} of animal wastes in the form of faeces and urine are deposited directly on the land by grazing cattle and sheep (EPA, 2003). As a consequence, agriculture in Ireland is estimated to be the source of 82% of the N and 97% of ammonia in Irish inland surface waters (Toner et al., 2005) and 81% of N_2O emissions to air (DAF, 2007; DEHLG, 2007).

1.4 Nitrates in groundwater and surface waters and their sources

The mean NO_3^- concentrations in groundwater in Ireland between 2007 and 2008 exceeded the Irish Threshold Value concentration of 37.5 mg NO_3^- L^{-1} at approximately 7% of all EPA monitoring locations (Lucey, 2009). Only 1% of these monitoring locations exceeded the drinking water Maximum Admissible Concentration (MAC) of 50 mg NO_3^- L^{-1}. The south east and south of the country have greater proportion of monitoring locations with elevated NO_3^- concentrations: 40-50% of the wells have concentrations of 10-25 mg NO_3^- L^{-1} for the 1995-2008 evaluation periods. The Irish EPA (McGarrigle et al., 2010) evaluated NO_3^- concentrations in groundwater in 2,681 individual monitoring samples at 211 monitoring locations between the year 2007 and 2009 and concluded the following:

- Nitrate concentrations greater than 37.5 mg L^{-1} NO_3^-, which is the 'Trigger Value' of the European Communities Environmental Objectives (Ground Water) Regulations 2010 (S.I. No. 9 of 2010), were recorded in 186 individual samples;

- Some 50 samples exceeded the Nitrate Directive (91/676/EEC, EC 1991) / Drinking Water (EU Drinking water Directive, 98/83/EC) MAC of 50 mg L^{-1} as NO_3^-;

- At ten (4.7%) of the monitoring locations, the mean concentrations exceeded 37.5 mg L^{-1} NO_3^-, while at two of these locations, the mean concentration exceeded 50 mg L^{-1} NO_3^-;

- Before 2006, a slight increase in NO_3^- concentrations has been detected over time. However, the 2007-2009 data indicated an overall decrease in NO_3^- concentrations, with a noticeable increase in the percentage of samples with concentrations less than 10 mg L^{-1} NO_3^-;

- River nitrate contamination surveillance in 2008 at 180 sites showed only 25% of sites with NO_3^- concentrations of 10-25 mg L^{-1} and only approximately 3% showed NO_3^- concentrations from 25-40 mg NO_3^- L^{-1} (Lucey, 2009). The rest of the sites had NO_3^- concentrations less than 10 mg NO_3- L^{-1};

- Nitrate concentrations in the lakes in Ireland are generally less than 2 mg L^{-1} NO_3^- in most of the lakes measured by McGarrigle (2010) with only very few groundwater wells having concentrations between 2 and 10 mg L^{-1} NO_3^-. None of the lakes monitored in 2008 (n=69) showed NO_3^- concentrations greater than 10 mg NO_3- L^{-1}. Mean NO_3^- concentrations in 92% of the lakes ranged from 0-2 mg NO_3- L^{-1}, whereas the rest of the lakes (8%) showed 2-10 mg NO_3- L^{-1};

- Nitrate monitoring during 2006-2008 in estuarine and coastal waters showed that 35 of 95 monitored water bodies breached the winter dissolved inorganic nitrogen (DIN) criterion of the European Communities Environmental Objectives (Surface Waters) Regulations 2009 (Lucey, 2009). In terms of the absolute concentrations, more than 50% of the estuaries and coastal waters had median values less than 1.0 mg N L^{-1}. Some of the coastal areas failed to comply with the environmental quality standard (EQS) established by the WFD for DIN status, Cork Harbour, Outer Cork Harbour and Malahide Bay;

- McGarrigle et al. (2010) analysed 2698 samples at 211 monitoring wells for ammonium (NH_4^+) and detected NH_4^+ concentrations greater than the drinking water MAC of 0.23 mg N L^{-1} in only 94 samples, and reported that 97% of the monitoring locations had mean concentrations less than the MAC.

Approximately 16.5 million tons of N was applied to European soils in 2003, with 7.6 million tons per year derived from animal husbandry (mainly cows, pigs, poultry and sheep) and 8.9 million tons from mineral fertilizers (Gumiero et al., 2011). In Ireland, groundwater is under increasing risk from diffuse (agricultural) and point sources (manure

and silage storage) and septic tank systems (Fenton, 2008). Nitrate concentrations higher than 10 mg NO_3^- L^{-1} are usually indicative of anthropogenic organic or inorganic inputs. Organic sources can include waste disposal (e.g., animal waste spreading or effluent from on site wastewater treatment systems); inorganic sources can include the spreading of fertilizer. Generally elevated NO_3^- concentrations are observed in monitoring points that are in close proximity to potential point source waste discharge; the spatial distribution of monitoring locations with elevated nitrate concentrations relates to areas with more intensive agricultural practices, suggesting that diffuse agricultural sources are the causes. The river sites showing higher NO_3^- concentrations have agricultural land in their immediate upstream catchment areas, suggesting that diffuse sources from agricultural activities are the main source of river NO_3^- contamination in Ireland. Atmospheric deposition can be an important source of NO_3^- which contains approximately 0.45 mg N L^{-1} as measured in Irish rainwater by Walmsley (2009).

1.5 Impacts of NO_3^- contamination in subsoil and groundwater

1.5.1 Human health concerns

Nitrate is not reported to be directly toxic to humans, but under strongly reducing conditions, such as those in the human gut, it transforms to nitrite (NO_2^-). 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 NO_3^- in drinking water has been associated with the risk of methaemoglobinaemia or 'blue baby syndrome' (Fan and Steinberg, 1996; WHO, 2004), an acute effect that is accentuated under poor sanitary conditions such as sewage contamination or dirty drinking vessels. High concentrations of NO_3^- (>23 mg N L^{-1}) have been shown to induce stomach cancer in animals, including mice and rats. However, epidemiological studies have not identified a link between exposure to NO_3^- and cancer in humans (Mason, 2002; WHO, 2004).

1.5.2 Environmental concerns

Increasing N export from landscapes to coastal waters has been implicated in coastal eutrophication and the development of hypoxic zones (e.g., in the Gulf of Mexico; Rabalais, 2002) and harmful algal blooms (Paerl et al., 2002). Excess NO_3^- concentrations can cause eutrophication, which enriches a water body by increasing levels of nutrients

such as N and P (Mason, 2002). There is still some debate over whether N alone is the main driver of these problems (Dodds, 2006), but there is no question that the increases in N loading represent a major perturbation of streams, rivers, estuaries, and coastal marine waters. Since the 1970s, NO_3^- contamination of groundwater has become a significant environmental problem, with many parts of the world now reporting groundwater NO_3^- pollution (EEA, 2000; Rivett et al., 2007; Roy et al., 2007; OECD, 2008). High nutrient levels affect biodiversity by favouring plants which need, prefer or can survive in nutrient-rich environments, and this can lead to excessive algal growth. Low oxygen levels caused by algal respiration or decay may 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. The full impact of eutrophication depends primarily on the balance between N and P concentrations in a water body. Where there is excess P but little N, small additions of NO_3^- can lead to changes in the trophic status. In the freshwater environment, excess NO_3^- affects oligotrophic waters (Mason, 2002) typically found in upland areas in the UK (Palmer and Roy, 2001). Nitrate imbalance in surface waters can lead to other detrimental effects including acidification. For example, high NO_3^- 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, mobilises heavy metals to produce toxic conditions in the water courses (Neal et al., 1992).

1.5.3 The economic involvement

The European Union has set the standard for NO_3^- in potable water at 11.3 mg N L^{-1} (50 mg N L^{-1}) (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^{-1}. The cost of removing NO_3^- from drinking water supplies to comply with drinking water standards is significant. In addition to the financial burden of treatment, water resources are lost, as boreholes with excessive NO_3^- concentrations are abandoned (Knapp, 2005). In the UK alone, the cost of treatment to ensure potable water supplies are below 50 mg NO_3^-

L^{-1} 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 NO_3^- water for blending becomes scarcer (Defra, 2006). Nitrogen pollution from farms, vehicles, industry and waste treatment is costing the EU up to £280bn (€320 bn) a year (BBC, 2011), where livestock is reported as the biggest source. Nitrate pollution as a diffuse source from agricultural practices is due to high N application to increase food production. Reduction of N input in the agricultural production systems can reduce economic losses to farmers, but appropriate management practices can increase production together with the reduction of N application. Therefore, an appropriate N management practice is crucial to maintain sustainable production with a substantial reduction of N inputs which eventually reduce cost for ecological and environmental protection.

1.6 Transport, leaching and biochemical transformation of NO_3^- in subsoil and groundwater

Nitrate is the most mobile form of N, and moves with percolating water from below the rooting zone to groundwater. Downward movement of water through the profile can cause NO_3^- to leach, with the magnitude being proportional to the concentration and water flux (Pierzynski et al., 2005). Prediction of potential solute attenuation by dispersion and advection through the unsaturated zone is a well-documented science (Fetter, 1999), although quantifying the constitutive relationships can be difficult and expensive. However, in the unsaturated zone, very detailed studies have demonstrated minor decreases in NO_3^- concentrations within infiltration water. Nonetheless, where it has been quantified, the losses are of the order of one to two per cent of the NO_3^- load in the infiltrating water (Buss et al., 2005). Therefore, it is unlikely that these processes offer an opportunity to significantly impact regional groundwater quality.

The processes controlling NO_3^- attenuation in the soil zone are well understood. However, for the environment *beneath* this zone, relatively little is known about the fate of NO_3^-. An understanding of the fate of NO_3^- in groundwater is vital for managing risks associated with NO_3^- pollution, and to safeguard groundwater supplies and groundwater-dependent surface waters. As nitrate-rich water flows through landscapes, it enters riparian wetlands and headwater streams, which can efficiently remove N (Peterson et al., 2001; Zedler, 2003).

1.7 N_2O as a greenhouse gas

Nitrous oxide, an intermediate product of denitrification, is one of the most important greenhouse gases. The atmospheric concentration of N_2O continues to rise at a rate of approximate 0.26% per year and reached a concentration of 319 ppb (10^{-9} mol mol^{-1}) in 2005 (IPCC, 2007). The science of global warming has reached a consensus on the high likelihood of substantial warming over the coming century and recent projections suggest that substantial future warming will occur if no abatement policies are implemented (Nordhaus, 2010). Globally, agricultural N_2O emissions increased by nearly 17% from 1990 to 2005 (IPCC, 2007) and total annual emissions of N_2O are estimated to be 17.7 Tg N (6.7–36.6 Tg N). Nitrous oxide is known to contribute to global warming (Duxbury and Mosier, 1993) and to the destruction of stratospheric ozone (Prather et al., 2001). The 100-year global warming potential of N_2O is about 300 and 23 times as strong as that of CO_2 and CH_4, respectively, and its half life is 114 years. Because of N_2O reaction with stratospheric ozone, the ozone concentration decreases and this may result in an increase in UV-radiation. Indirect N_2O emissions via groundwater, drainage and estuaries account for an important component (10%) of global N_2O budget. However, no national indirect N_2O emissions inventory is available in Ireland, so far, hence the use of the IPCC default emission factor, which has high uncertainty.

1.8 Legislative frameworks for water quality protection

As previously stated, the EU and WHO have both set the standard for NO_3^- in potable water at 11.3 mg N L^{-1} (50 mg NO_3^- L^{-1}) (Drinking Water Directive 98/83/EC; WHO, 2004). A guide level of 25 mg NO_3^- L^{-1} (5.6 mg N L^{-1}) is specified in the EU Drinking Water Directive (EC, 1998), which is recommended as an indication of contamination (McGarrigle et al., 2002). A NO_3^- value for eutrophication in Irish estuaries and coastal waters is defined as 11.6 mg NO_3^- L^{-1} (2.6 mg N L^{-1}) should be referenced when assessing water quality known to cause eutrophication conditions. Nitrite is toxic to aquatic animals and the EU guideline concentration for NO_2^- in rivers supporting salmonid fish is 0.01 mg N L^{-1}, while for cyprinids it is 0.03 mg NO_3^- L^{-1} (Freshwater Fish Directive, 78/659/EEC). Considering the contributions of agricultural practices, the Kyoto Protocol imposed the industrialized and European countries to reduce emissions to an average of 5% against 1990 levels over the five-year period 2008-2012.

1.8.1 Water Framework Directive

On 22 December 2000, the European Union (EU) passed a directive establishing a framework for Community action in the field of water policy, commonly known as the WFD. The WFD aims at protecting and enhancing all waters – groundwater, rivers, lakes, transitional waters (estuaries) and coastal waters – and includes terrestrial ecosystems and wetlands directly dependent on aquatic systems. The WFD is concerned, *inter alia*, with the protection of the aquatic ecosystem, prevention of further deterioration and, where necessary, its restoration, to achieve conditions (good ecological status) in all waters that are no more than slightly degraded from those of the natural or reference state. The default objectives of the WFD include the prevention of any deterioration in the existing status of waters, including the specific requirements to maintain 'high status' where it exists and to ensure that all waters achieve at least 'good ecological status' by 2015. The definition of good status in the case of surface waters is based on both ecological status, and the natural chemical and physical characteristics; and chemical status, which, in the context of the directive, refers to a number of specified toxic and/or bioaccumulative substances. In the case of groundwaters, good status relates to the natural chemical composition of the water and to these same chemical substances as well as to quantitative status.

1.8.2 Nitrates Directive

Agriculture remains a major source of water-related problems, and farmers need to continue to adopt more sustainable practices. The European Union Nitrates Directive (EC, 1991) aims to protect water quality across Europe by preventing nitrates from agricultural sources polluting groundwater and surface waters and by promoting the use of good farming practices. The target value of NO_3^- and NO_2^- in water should remain below the standards of 11.3 and 0.2 mg N L^{-1}, respectively. Under the Directive, which is now subsumed into the WFD, all Member States have to analyse their waters' NO_3^- concentration levels and trophic state. Still around 33% of monitoring stations in European rivers and lakes show signs of eutrophication, as well as some coastal waters (EU Nitrates Directive report, 2010). Ultimately, the aspects of the WFD relating to groundwater have been transposed into Irish national legislation through the 'Groundwater Regulations' (European Communities Environmental Objectives (Groundwater) Regulations, 2010: SI 9 of 2010) under which the threshold at which NO_3^- concentration posed a risk to groundwater bodies was set at 37.5 mg L^{-1}.

1.8.3 Kyoto Protocol

The Kyoto Protocol is the first international agreement in which many of the world's industrial nations concluded a verifiable agreement to reduce their emissions of greenhouse gases: CO_2, CH_4, and N_2O in order to prevent global warming. The major feature of the Kyoto Protocol is that it sets binding targets for 37 industrialized countries and the European community for reducing emissions. Ireland's commitment on GHGs under the Kyoto Protocol, as determined by decision 2005/166/EC, is to limit the increase in emissions in the 2008-2012 commitment periods to 13% above the base year emissions. The baseline emissions total for Ireland is calculated as the sum of CO_2, N_2O, and CH_4 emissions in 1990.

1.9 Need for this research

Elevated NO_3^- concentrations in groundwater may lead to the derogation of precious aquifer resources and the eutrophication of surface waters. Understanding of processes controlling the natural attenuation of NO_3^-, which may lead to risk reduction, is critical to the implementation of WHD and Nitrate Directive. Apart from physical attenuation processes such as dispersion, the attenuation of NO_3^- in groundwater may occur via denitrification, DNRA and other pathways. Denitrification requires all the following conditions to be met:

- The presence of NO_3^-, denitrifying bacteria and electron donor (organic carbon, reduced iron and/or reduced sulphur);
- Anaerobic conditions;
- Favourable environmental conditions (e.g. temperature, pH, other nutrients and trace elements).

The biogeochemical processes controlling NO_3^- attenuation under existing land use or hydrogeochemical environments are not well defined and so warranted further research. As such, we need to understand the following:

- The major pathways acting on natural NO_3^- attenuation in the specific land use system;

- The environmental factors that control natural attenuation of NO_3^-;
- The NO_3^- removal capacity and indirect N_2O emissions to the atmosphere; and
- The agricultural practices in grassland and tillage farming that should be adopted to keep NO_3^- concentrations below the target of the WFD and to reduce the indirect N_2O emissions to the atmosphere.

Within these contexts, the following research areas are considered crucial to improve our understanding concerning the knowledge gaps:

- Extending nitrate attenuation study from shallow to deeper groundwaters;
- Study of the distribution and availability of C as electron source for microbial reactions in different groundwater zones under varying hydrogeological settings and land uses;
- Evaluation of the occurrence of heterotrophic and autotrophic denitrification individually and within multiple electron donor systems;
- Environmental conditions that control denitrification processes e.g., dissolved oxygen concentration, redox potential; hydrologic conditions- residence time, depth of unsaturated zone and water table fluctuations; pH, and inhibitions by metals;
- Quantification of denitrification rates that may be scaled from laboratory scale to agricultural catchment, national and continental scales;
- In situ measurements of NO_3^- depletion in soil and groundwater zones by intensive monitoring of groundwater samples and using definitive methods to determine the contribution of denitrification. Detailed hydrogeological and geochemical studies and an investigation of the relationships between varying parameters identify potential zones for NO_3^- depletion by denitrification;
- Temporal and spatial variabilities in factors controlling denitrification and rates of denitrification across land use and landscape settings;

- Comparing and improving methodologies for measuring in situ denitrification;
- Identification of major NO_3^- removal pathways in groundwater e.g., denitrification, DNRA etc;

- Data for national GHG inventories requires the emissions via groundwater

1.10 Objectives of the research

The major objectives of the present research project are to:

1. Quantify subsoil and groundwater denitrification rates and N_2O/N_2O+N_2 ratios in a range of hydrogeological settings and land use;

2. Investigate the major NO_3^- removal pathways in subsurface environments and to elucidate how these are controlled by the site hydrogeology and geochemistry;

3. Quantify actual and potential subsoil and groundwater denitrification in a number of agricultural systems;

4. Estimate the NO_3^- and dissolved N_2O, CH_4 and CO_2 distribution in subsurface environments, their delivery through the landscape towards the surface waters;

5. Provide the indirect N_2O emissions (via groundwater denitrification) data for national GHG inventories;

6. Assess relevant groundwater quality in respect of different organic, inorganic and metal contaminants.

CHAPTER 2. BIOCHEMICAL TRANSFORMATIONS OF N IN SUBSOILS AND GROUNDWATER

2.1 Overview of this chapter

This chapter reviews available information on N sources in the environment, its transformations, physical and biochemical retention and factors affecting denitrification processes. A general introduction of this chapter is presented in section 2.2. The methodological advancement for denitrification measurement in subsoil and groundwater, as well as the methods used in this project was presented in section 2.11.

2.2 Introduction

The WFD, Directive 2000/60/EC, was adopted in 2000 as a single piece of legislation covering rivers, lakes, groundwater and transitional and coastal waters. Its objectives include the attainment of 'good ecological status' in water bodies that are of lesser status at present and retaining good status or better where such status exists at present. Groundwater is an important water resource in the Republic of Ireland. Approximately 26% of the public and private drinking water supply in Ireland is provided by groundwater (Lucey, 2009). Understanding of the underlying causes of ground and surface water NO_3^- contaminations and using this information for management practices requires a sound knowledge of the terrestrial and aquatic N cycle. The soil N cycle is an assembly of input and output fluxes, N-pools and internal fluxes. Mineralization, nitrification and denitrification processes control transformations of N between the various pools (Brady and Weil, 2002). Most of the N applied to the field is generally converted to NH_4^+-N and finally to NO_3^--N (Defra, 2003), which is then available for leaching to soil, groundwater and consequently to nearby surface waters.

Physical solute transport processes, such as dispersion and advection, are unlikely to offer an opportunity to significantly attenuate NO_3^- and to impact regional groundwater quality (Buss et al., 2005). Therefore, an understanding of the fate of NO_3^- in subsoil and groundwater is vital for managing risks associated with NO_3^- pollution, and to safeguard groundwater supplies and groundwater-dependent surface waters (Kellogg et al., 2005). Up to 75% of the N added to a landscape may be removed before reaching marine ecosystems

(Howarth et al., 1996). Unfortunately, very little is known about the fate and transformations of loaded N and its movement from landscape to groundwater and surface waters, which has led huge discrepancies in the N balance estimations. The various transformations and eventual fate of this N as it is carried along hydrological flow paths is a problem that has been of interest to scientific and management communities. The current consensus is that the disappearance of N is due largely to biological transformations, since increased N storage cannot explain most of the "missing N" (Howarth et al., 1996). New research has pointed to the importance of processes that remove NO_3^- in freshwater ecosystems, including denitrification, DNRA (Tiedje, 1988), anammox (Jetten, 2001), denitrification coupled to sulphide oxidation (Brunet and Garcia-Gil, 1996; Otte et al., 1999), and reduction of NO_3^- coupled to abiotic or biotically mediated oxidation of iron (Davidson et al., 2003; Weber et al., 2006). Much work is needed to understand where and when these pathways are prevalent in ecosystems and the driving processes controlling these pathways (Rivett et al., 2008).

The organisms capable of denitrification are ubiquitous in surface waters, soil and groundwater (Beauchamp et al., 1989) and 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). The most important denitrification pathways are i) organo-heterotrophic denitrification, where organic substances serve as electron donor, and (ii) litho-autotrophic denitrification, where reduced iron Fe (II) or reduced sulphur compounds act as electron donor. Further research is necessary to improve current understanding on the influences of organic carbon, sulphur and iron electron donors, influences of environmental conditions, and improved quantification of denitrification rates in the laboratory and field (Buss et al., 2005).

2.3 Nitrate Flow Pathways

The most common contaminant identified in groundwater is dissolved N in the form of NO_3^-. Although nitrate is the main form in which N occurs in groundwater, dissolved N also occurs in the form of NH_4^+, ammonia (NH_3), NO_2^-, nitrogen (N_2), N_2O and organic N (i.e. N that is incorporated in organic substances). Nitrate in groundwater generally originates from NO_3^- sources on the land surface, in the soil zone, or in the shallow subsoil zone where N-rich wastes may occur. In some situations, NO_3^- that enters groundwater

systems originates as NO_3^- in wastes or fertilizers applied to the land surface. A schematic flow path of NO_3^- from its different sources to groundwater is shown in Figure 2.1.

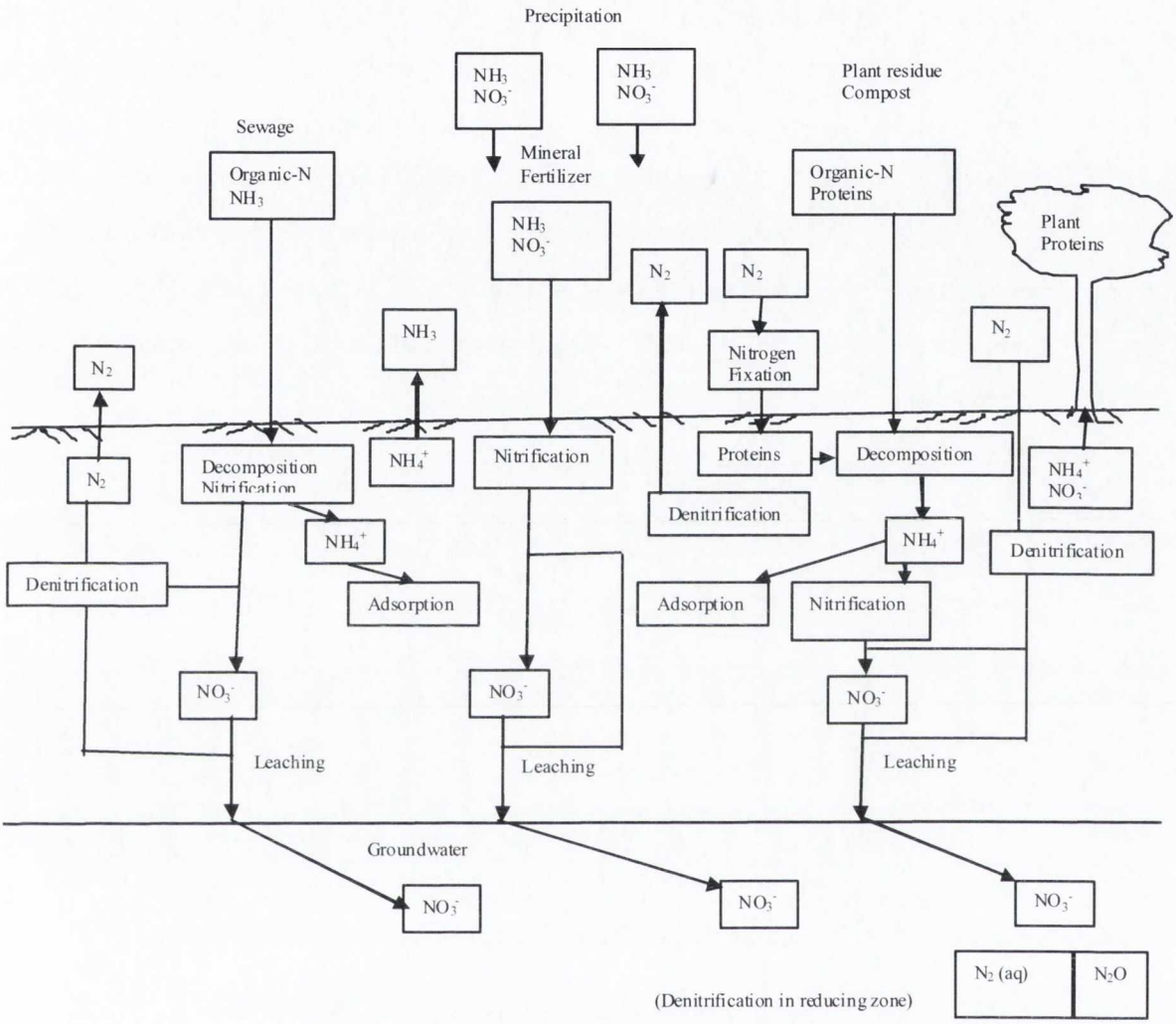


Figure 2.1 Sources and pathways of nitrogen in subsurface environment (after Freeze and Cherry, 1998)

2.4 Sources of NO_3^- in subsoils and groundwater

2.4.1 Fertilizers

Agricultural activities are probably the most significant anthropogenic sources of NO_3^- contamination in groundwater (Oyarzun et al., 2007). Nitrogen can be imported as manure, dairy washings, and animal excretions or as sewage press cakes; in modern agriculture however, most N is imported as mineral fertilisers. The EPA (2008) summarized how the use of artificial fertilisers affects Irish agriculture between 1990 and 2006 (Figure 2.2). Lalor et al. (2010) reported the fertilizer N application in Irish grass and crop lands

between 1995 and 2008 showing a declining trend of fertilizer applications with the highest at 145 kg ha^{-1} in 1999 and the lowest at 86 kg ha^{-1} in 2008 for grassland. The authors also reported a decreasing trend in fertilizer applications for cereal crops, being the highest at 160 kg N ha^{-1} in 2000 and the lowest at 127 kg ha^{-1} in 1999. Foster (2000) showed a 3-fold increase in food production has been accompanied by a 20-fold increase in the use of fertilisers. The remainder of N has, therefore, been lost to the atmosphere by denitrification, leached to groundwater and surface waters as nitrate, or remains stored as a source of NO_3^- in the unsaturated zone. Most of ammonium is converted to NO_3^- in the soil zone. Depending on the soil type and the timing of the application, Defra (2000) estimated that typically 5 to 50% of this N is mineralised and can be made available for plant growth and leaching.

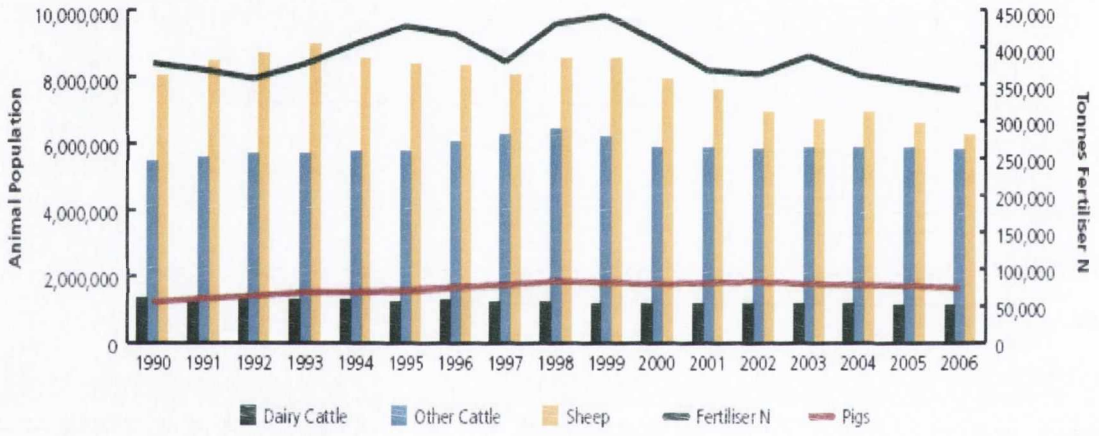


Figure 2.2 Use of inorganic N fertilizers in Ireland (EPA Report, 2008)

2.4.2 Organic sources of N

Animal wastes, farmyard wastewaters (soiled water and dirty water), animal slurry and on-site wastewater treatment systems are important sources of N in groundwater. Dirty wash water is potentially and probably an important conduit for nutrient losses to water from dairy farms (Humphreys et al., 2003). In dairy farming systems, each cow produces 85 kg ha^{-1} organic N where stocking rates are 2.5 cows ha^{-1}. In cattle, pig and sheep faeces the concentrations of N ranged from 0.2 to 0.5% of fresh weight but >90% of it is in organic forms and >70% is in insoluble forms (Chambers et al., 2001). Silage effluents are reported to contain 37 mg N L^{-1} as NO_3^- together with other forms of reactive N, mainly NH_4^+ (Mulqueen et al., 1999). Nonetheless, the mean annual N surpluses of 232 kg N ha^{-1} as

estimated by Treacy et al. (2008) from 21 farms with N use efficiency of 20%, indicated the serious threat to ground and surface water NO_3^- contamination.

2.4.3 Point sources of N

Fertilizer production industries and wastewater treatment plants can be potential sources of NO_3^-. 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). Pieterse et al. (2003) suggested that point sources are the main contributor to TN fluxes, although they affect few tributaries only. The sanitary sources often discharge N in organically-bound forms under reduced conditions; these are usually quickly mineralised to ammonium, and under aerobic conditions this can be oxidised to NO_3^- (Buss et al., 2005). Fertilizer manufacturing plants can sometimes be a source of N which can discharge ammonium and/or NO_3^-. Septic tanks contains human wastes and domestic waste waters connected a primary sedimentation tank, removing suspended solids from wastewater with limited amount of anaerobic digestion, but in filter areas the waste water is purified, undergoing surface infiltration, the effluents eventually percolate towards groundwater (Rodgers et al., 1998). Wakida and Lerner (2005) noted the concentrations of TN in septic tank ranges from 25 to 60 mg N L^{-1}, up to 80% of it is inorganic N, mainly NH_4^+/NH_3, whereas nitrate is marginal (Rodgers et al., 1998).

2.4.4 Change in land use

Anthropogenic nitrate sources are septic systems, sanitary sewage effluent releases, domestic animal wastes, and home and farm usage of N fertilizer. Agriculture contributes to the major part of NO_3^- input to groundwater, accounting for 82% of all inputs of NO_3^- (EPA, 2002). Swapping between land use systems and or intensifying a specific land use system influences the nutrient content in soil and groundwater, and consequently nearby surface waters. Ploughing exposes soil-bound NH_4^+ compounds and organically-bound N to the atmosphere. These are mineralised to NO_3^-, which is readily leached by rainfall runoff and infiltration. Generally grassland agriculture requires higher NO_3^- input than arable land. Therefore, N input, production, land management and the transformations in soil and subsoils may vary with land use and eventually can discriminate N delivery to

groundwater e. g., 93 kg $ha^{-1}y^{-1}$ in ploughed grass (DoE, 1988), 36 kg $ha^{-1}y^{-1}$ in grass ley (McLenaghen et al., 1996), 72-142 kg N $ha^{-1}y^{-1}$ in temporary leguminous pasture (Francis, 1995).

2.4.5 Nitrogen deposition from the atmosphere

Atmospheric deposition can be an important non-point source of NO_3^- on land surface which subsequently delivered to groundwater (Kaushal et al., 2011). Atmospheric N originates from a variety of natural and anthropogenic sources, and is deposited on land under both wet and dry deposition. Nitrogen in atmospheric deposition originates primarily from the combustion of fossil fuels, such as coal and oil. Atmospheric deposition may be in a wet form as rain, snow, hail, fog, and freezing rain or in a dry form as particulates, gases, and droplets. However, automobiles, trucks, buses, and other forms of transportation account for approximately 38% of N emissions (Puckett, 1994). Natural sources include HNO_3 created from N gas and water vapour by lightning, and natural NH_3 emissions from rotting vegetation and manure (Buss et al., 2005). Walmsley (2009) measured 0.45 mg N L^{-1} in rainwater during 2007 in Oak Park, Co. Carlow, Ireland and suggested that rainwater is a significant source of NO_3^-. Goulding et al. (1990) estimated TN deposited by atmospheric deposition amounting to 35 to 40 kg N ha^{-1} y^{-1} on arable land in south and east of England, of which approximately 10% was leached out during winter (MAFF, 1995). Rainfall deposition of mean NO_3^- and ammonium respectively were 0.35 and 0.42 mg N L^{-1} in the UK (Hayman et al., 2001).Wakida and Lerner (2005) estimated such deposition to be as high as 10 to 20 kg N ha^{-1} y^{-1} in UK.

2.4.6 Geogenic sources of N

Nitrate from geogenic sources include desert-derived deposits containing natural perchlorate caliche in some desert areas. Geologic N is defined as N contained in rock or sediment. Organic matter-rich sediments contain relatively high concentrations of organically bound N, which is mineralised to NH_4^+ as the sediment undergoes diagenesis to form a sedimentary rock (Rodvang and Simpkins, 2001). When this NH_4^+ is converted to NO_3^- by nitrification, it can produce high levels of NO_3^- that are entirely natural (Buss et al., 2005). An organic-rich carbonaceous siltstone and a fine-grained calcareous sandstone showed N concentrations of 530 and 670 ppm, respectively, in Utah (Lowe and Wallace,

2001). Rodvang and Simpkins (2001) showed that geological NO_3^- in North American aquitards is, on average, present at a much higher concentration than that from agricultural pollution: 164 mg N L^{-1} and 32 mg N L^{-1} respectively. In Ireland, there are no available data on the geogenic NO_3^- content, but some geogenic NO_3^- can exist in the Northern part of the country (pers. comm., Dr. T. Hunter, GSI).

2.5 The N cycle

The N cycle is closely related to water movement in the continuum of groundwater- soil-plant and atmosphere. The global N cycle plays a vital role in the functioning of all ecosystems, and influences every aspect of the biosphere and the climate (Stark and Richards, 2008). Nitrogen from natural sources (rainfall and geological formations) and from anthropogenic sources (forage, and pastoral agriculture, fertilisers and waste disposal) undergoes mineralization, immobilization, fixation, nitrification, and denitrification (Table 2.1). As NO_3^- moves through the N cycle, an atom of N may occur in many different organic and inorganic chemical forms, each performing an essential role in the ecosystem. The N cycle involves the following reactions (Brady and Weil, 2002):

Fixation: Despite N is freely available in the atmosphere the very stable triple bond of the dinitrogen (N_2) molecule requires considerable energy to break. This complex process is carried out by N-fixing bacteria present in the soil/sediments. This can be accomplished by a limited number of bacteria (e.g., *Rhizobium)* that tend to be symbiotic with plants such as legumes, where the higher plant supplies energy for the reaction from photosynthesis or free living (e.g., *Azotobactor, Klebsiella, Clostridium etc.*). Anthropogenic N fixation occurs in N fertilizer, and energy production and cultivation of leguminous crops in agricultural systems with a total human-induced global N-fixation of about 150 Tg y^{-1} (Galloway et al., 1995). In fixation reaction, free N is converted to ammonia, which may then be assimilated by the plant. The reaction can be shown as:

$$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2 \quad \text{(Eqn. 2.1)}$$

The above reaction is performed exclusively by prokaryotes, which have an enzyme complex termed nitrogenase.

Mineralisation (ammonification): Most of the N in soils is present within organic compounds that make it insoluble and unavailable for use by higher plants. Much of the N is present as amine groups ($R–NH_2$), in proteins or as part of humic compounds (Buss et al., 2005). Soil microorganisms convert these to simpler amino-acids, then to NH_4^+. However, this multi-step process depends on the environmental and soil mineralogy factors which determine the rate of N mineralization in the soil and thus the amount mineralized over time. Soil temperature, aeration and moisture content have a strong effect on N mineralization reactions due to their connection to the activities of soil microbes involved in mineralization. The ammonification reaction can be shown as:

$$R\text{-}NH_2 + H_2 \rightarrow NH_4^+ + \text{Energy} \qquad \text{(Eqn. 2.2)}$$

Nitrification: Nitrification is the oxidation of ammonium to NO_2^- and then to NO_3^-. This process is carried out by a small group of microbes e.g., *Nitrosomonas, Nitrospira* convert NH_4^+ to NO_2^-, and *Nitrobacter* convert NO_2^- to NO_3^-. This process occurs under aerobic conditions (EA, 2002), but many other factors control the whole process: the nitrification process is enhanced by temperature (Cookson et al., 2002; Harrison et al., 1994), by a coarse well oxygenated soil that guarantees adequate soil moisture (Jarvis et al., 1996), and by a succession of short wet and dry event (Haynes, 1986), but is curtailed below pH 6 (Paul and Clark, 1989), and inhibited at greater than pH 8 (Whitehead, 1995).

Reaction: $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{Energy};$ (Eqn. 2.3)

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{Energy} \qquad \text{(Eqn. 2.4)}$$

Denitrification: Denitrification is a microbially mediated multi-step process which converts NO_3^- to N. The intermediate products of denitrification are NO_2^-, nitric oxide (NO) and N_2O. Nitrous oxide is an obligate intermediate product of denitrification which is a potent GHG, but the stable end product of denitrification is environmentally benign. Denitrification is a microbially mediated process where NO_3^- is used as a terminal electron acceptor to produce N_2 or N_2O (Starr and Gillham, 1993). Denitrification occurs under anaerobic/suboxic conditions where electron donors are available.

Assimilation (immobilisation): This process is opposite of mineralisation and describes the conversion of ammonium and NO_3^- (formed by mineralization and nitrification) into organic forms and ultimately biomass. The extent of immobilization is determined by the

amount and quality of C and N in soils and sediments, but in groundwater, dissolved C and N is generally low. Mineralization, nitrification and immobilization occur simultaneously resulting in the transformations of N from organic to inorganic forms and vice versa. Therefore a net mineralization term is applicable to determine the amount of NO_3^- and NH_4^+ readily available to be fed from soil to groundwater, and then groundwater to receptors.

Table 2.1 Summary of N cycle, biochemical reactions involved and brief descriptions (after Hauck and Tanji, 1982)

Transformation	Chemical Reaction	Description
N-fixation	$0.5\ N_2 \rightarrow R\text{-}NH_2$	Plants and some microorganisms use N_2 from the air and convert it to ON in a symbiotic relationship with microbe
N-mineralization	$R\text{-}NH_2 + H_2O + H^+ \rightarrow R\text{-}OH + NH_4^+$	Transformation of organic N to inorganic N (NH_4^+) as microorganisms decompose organic matter
N-immobilization		Transformation of inorganic N into organic N as microorganisms incorporate N into their structure of humus during decomposition
From NO_3^-	$NO_3^- + 2e^- \rightarrow NO_2^- + 6e^- \rightarrow NH_4^+$	
From NH_4^+	$NH_4^+ + R\text{-}OH \rightarrow R\text{-}NH_2 + H_2O + H^+$	
NH_3 volatilization		Loss of ammonia from soil water to air
first stage (in water)	$NH_4^+ \rightarrow NH_3\ (aq) + H^+$	
from water to air	$NH_3\ (aq) \rightarrow NH_3\ (air)$	
Nitrification		Transformation of NH_4^+ to NO_2^- and NO_3^- by microorganisms
By *nitrosomonas*	$NH_4^+ + 1.5\ O2\ (aq) \rightarrow NO_2^- + H_2O + H^+$	
By *nitrobactor*	$NO_2^- + 0.5\ O2\ (aq) \rightarrow NO_3^-$	
Denitrification		Transformation of NO_3^- to N gases
to N_2 (g)	$NO_3^- + 1.25\ (HCHO) \rightarrow 0.5\ N_2 + 0.75\ H_2O + 1.25\ CO_2 + OH$	
to N_2O	$NO_3^- + (HCHO) \rightarrow 0.5\ N_2O + 0.5\ H_2O + CO_2 + OH^-$	

2.5.1 Denitrification

Denitrification is a multi-step process whereby nitrate is converted, via a series of microbial reduction reactions, to nitrogen gas (Figure 2.3). It can also be reduced to NO_2^- and N_2O by abiotic reactions, but in the subsurface these reactions are minor in comparison with biological denitrification (Buss et al., 2005). In denitrification, reduction of NO_3^- or NO_2^- to elemental N takes place by a series of bacterial processes (Kristiansen and Schaanning, 2002). The denitrifiers tend to be ubiquitous in surface water, soil and groundwater (Beauchamp et al., 1989). In general, the absence of oxygen and the presence of organic carbon, reduced sulphur or iron facilitate denitrification (Buss et al., 2005).

$2NO_3^-$ → $2NO_2^-$ → $2NO$ → N_2O → N_2

Nitrate ions (+5) Nitrite ions (+3) Nitric oxide (+2) Nitrous oxide (+1) Dinitrogen (0)

Figure 2.3 Denitrification reaction chain. Numbers in brackets refer to the valence state of the nitrogen at each step (after Brady and Weil, 2002).

Biological denitrification can best be described by the following reaction:

$$4NO_3^- + 5CH_2O + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O \qquad \text{(Eqn. 2.5)}$$

The NO_3^- 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):

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \qquad \text{(Eqn. 2.6)}$$

Denitrification can be an important process of groundwater NO_3^- removal as its effectiveness has been highlighted recently by other researchers (Seitzinger et al., 2006; Groffman et al., 2006; Rivett et al., 2007; Domagalski et al., 2008).

2.5.1.1 Process based nature of denitrification

Redox chemistry and denitrification

Young and Briggs (2007) suggested that redox conditions in groundwater significantly influences NO_3^- distribution along groundwater flow paths due to its associated impacts on

denitrification. Denitrifiers in aquifers obtain energy from the oxidation of organic C or inorganic compounds e.g., reduced iron, manganese and sulphur minerals. Bacteria that use organic carbon as the energy source also tend to use it as a source of cellular C (heterotrophs), while those that use inorganic compounds will normally use inorganic carbon (mainly from HCO_3^-) for cell construction (autotrophs) (Buss et al., 2005). Assuming that the electron donor is organic C, there is an ideal sequence of redox reactions based on the energy available. In this sequence, organic C will first be degraded by the electron acceptors in the following order: O_2, NO_3^-, manganese oxide, iron oxide, and then sulphate (Puckett and Cowdery, 2002). Denitrification in groundwater may be related to DOC that is carried into the saturated zone, but in most aquifers, it is coupled with oxidation of solid phases (organic C, reduced Fe, Mn and S minerals) (Seitzinger et al., 2006). 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). Denitrification is central to the N cycle (illustrated in Figure 2.4) with respect to the subsurface groundwater environment and involves the reduction of NO_3^- via a chain of microbial reduction reactions to N_2 (Knowles, 1982). The NO_3^- reduction reaction can be written as a half equation that illustrates the role of electron transfer in the process (non-specific to the electron donor) (Tesoriero et al., 2000):

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \qquad \text{(Eqn. 2.7)}$$

Redox potential (Eh) measures the availability of electrons for transfer between chemical species and is one of the most important measures used to characterize groundwater systems (Thyalakumaran et al., 2008). 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). Brettar (2002) measured Eh value of 10-300 mV under denitrifying conditions in floodplain soils where Eh decreased with increasing NO_3^- reduction. While Eh can be measured using different redox probes, redox conditions in an aquifer can best be defined by simultaneously measuring several redox couples e.g., DO concentrations (indicates oxic zone), ferrous iron and manganese, or other reduces species (indicate reduced zone) (Postma et al., 1991).

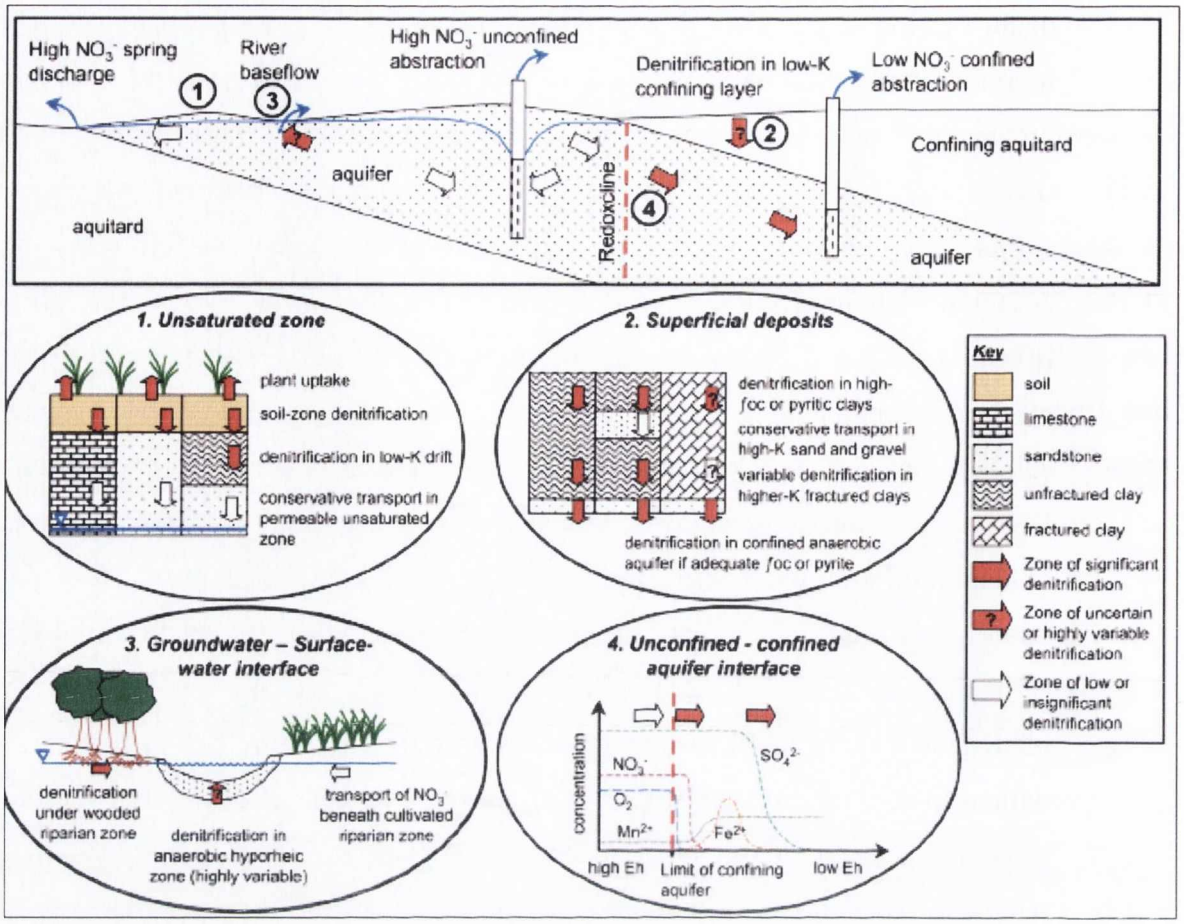


Figure 2.4 Conceptual model of denitrification occurrence in the subsurface environment (Rivett et al., 2008).

2.5.2 Dissimilatory NO_3^- reduction to ammonium (DNRA)

The DNRA is an anaerobic process where NO_3^- is transformed to NH_4^+ which can remain in that form only until it has contact with an aerobic environment (Korom, 1992; Tesoriero et al., 2000) after which it is oxidized to NO_3^- (Thayalakumaran et al., 2008). The DNRA reaction can be shown as (Robertson et al., 1996):

$$2H^+ + NO_3^- + 2CH_2O \rightarrow NH_4^+ + 2CO_2 + H_2O \qquad \text{(Eqn. 2.8)}$$

DNRA is thought to be favoured by a high ratio of available C to N ratio and occurs at low level of Eh (Tayalakumaran et al., 2008). The differences between denitrification and DNRA may be due the availability of organic matter, because DNRA is the favoured at high C: N ratio and denitrification is favoured when carbon supplies are limiting (Korom, 1992; Kelso et al., 1997). The fermentative bacteria which carry out DNRA are obligate anaerobes (Hill, 1996) and so cannot occupy all the niches that denitrifiers can (Buss et al.,

2005). Little is known about the eventual fate of the NO_3^- that is converted to NH_4^+ via DNRA pathways. In recent years N cycling studies have increasingly investigated DNRA in various ecosystems and it is time to revisit this often forgotten process to explore its importance in N cycling in terrestrial ecosystems (Rütting et al., 2011). However, DNRA can be a significant pathway of nitrate reduction which impacts in the ecosystems should be evaluated.

2.5.3 Abiotic denitrification

The reduction of NO_3^- coupled to iron cycling is thought to take place through both biotic and abiotic pathways (Davidson et al., 2003; Weber et al., 2006). Postma et al. (1991) concluded that this reaction would only remove a major proportion of NO_3^- from groundwater in areas with low NO_3^- inputs. Another abiotic reaction has been proposed, in which NO_3^- is reduced to NO_2^- by reaction with Fe or Mn and the nitrite binds with organic substances to produce DON; Davidson et al., 2003). Evidence for this reaction was discovered recently in forest soils (Dail et al., 2001), but it has not, so far, been shown to occur in agricultural and aquatic ecosystems. Alternatively, microbes can mediate NO_3^- reduction coupled to iron oxidation in aquatic ecosystems (Weber et al., 2006). The controls on the process remain poorly understood, although it may be important in areas of high reduced iron and a limited supply of organic carbon (Weber et al., 2001).
The reactions can be shown as:

$$10\ Fe^{2+} + 2\ NO_3^- + 14\ H_2O \rightarrow 10\ FeOOH + N_2 + 18\ H^+ \qquad \text{(Eqn. 2.9)}$$

$$15\ Fe^{2+} + NO_3^- + 13\ H_2O \rightarrow 5\ Fe_3O_4 + N_2 + 28\ H^+ \qquad \text{(Eqn. 2.10)}$$

2.5.4 Anammox (anaerobic ammonium oxidation)

Anammox is a chemolithoautotrophic process by which NH_4^+ is combined with NO_2^- under anaerobic conditions, producing N_2. The NO_2^- is derived from the reduction of NO_3, possibly by denitrifying bacteria, and anammox therefore contributes to permanent NO_3^- removal. The process was discovered in a wastewater treatment system in the 1990s, and since then, studies have shown it to occur in anoxic wastewater, oxygen depleted zones of the ocean, temperate shelf sediments, sea ice, and cold Arctic shelf sediments (Rysgaard and Glud, 2004; Rysgaard et al., 2004). It has also recently been reported in one freshwater

ecosystem (Schubert et al., 2006). Scientists still know relatively little about the bacteria that carry out anammox, and no pure cultures exist (Strous et al., 2006). Thus, anammox may be most important in ecosystems with limited labile carbon or an excess of N relative to carbon inputs. This may include substantial parts of the pelagic ocean and continental shelves (Dalsgaard et al. 2005). The only study to date on anammox in freshwaters was conducted in Lake Tanganyika, where Schubert et al. (2006) found that 7–13% of the N_2 production was derived from anammox.

The anammox reaction can be shown as:

$$NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O \qquad \text{(Eqn. 2.12)}$$

2.5.5 Assimilation to microbial biomass

Conversion to biomass growth can become an important mechanism for N removal. Kelso et al. (1999) showed that in the presence of some organic substrates, up to 50% of N 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). For example, a kilogram of hydrocarbon contaminated aquifer may contain 2.5 x 10^{10} bacterial cells (Holm et al., 1992). Furthermore, rapid bacteria die-off may often be anticipated and lead to N release as ammonium back into groundwater.

2.5.6 Transformation products of NO_3^- in subsurface environments

2.5.6.1 Nitrite (NO_2^-)

In particular, the action of the NO_2^- reductase enzyme is more sensitive to oxygen concentrations than that of NO_3^- reductase (Hochstein et al., 1984; Korner and Zumft, 1989). Given the difference in energy available forms, the reduction reaction means that NO_3^- is used by denitrifiers preferentially to NO_2^-, even when both enzymes are present. A build-up of NO_2^- may then occur due to the time lag between the onset of NO_3^- reduction and the subsequent onset of NO_2^- reduction (Betlach and Tiedje, 1981; Gale et al., 1994). Nitrite also readily reacts with dissolved organic compounds to form DON compounds

(Davidson et al., 2003), especially in low pH where HNO_2 is the key reactant (Buss et al., 2005).

2.5.7 Nitric oxide and nitrous oxide (NO and N_2O)

Nitric oxide (NO) and N_2O are formed during denitrification but, in favourable conditions, transform rapidly to benign N_2. Both NO and N_2O contribute to acid rain, and contribute to global warming; N_2O also destroys ozone in the upper atmosphere (Prather et al., 2001). Free NO is rarely observed because it transforms to N_2O rapidly under typical environmental conditions. During denitrification both production and reduction of N_2O can take place simultaneously and significant amounts of N may also be lost to the atmosphere as N_2 (Meijide et al., 2010) because N_2O is short lived (Scolefield et al., 1997). When oxygen levels are very low, N_2 is the end product of denitrification process; but, where oxygen levels are more intermediate or variable, the reactions may stop with the formation of NOx (Brady and Weil, 2002). Very high NO_3^- concentrations or low pH values also arrest denitrification at the N_2O stage (Buss et al., 2005). 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 N_2 in anoxic benthic sediments. Estimations of indirect N_2O emissions from groundwater are highly uncertain because the controls on both N_2O production and consumption in groundwater are not well understood (Clough et al., 2007). IPCC set a default value for indirect N_2O emissions from groundwater of 0.0025 which have a high uncertainty (ranged 0.0005-0.025). In contrast with direct emissions, there are only very few data available to validate the IPCC emission factors (Groffman et al., 2002; Weymann et al., 2008).

2.5.8 Dinitrogen gas (N_2), environmentally benign N

At the stable end point of the denitrification chain, evolved nitrogen can be obscured by atmospheric N, especially in shallow systems. Past research has been directed to NO and N_2O because of their potential connection to global warming, but interest in these pressing environmental concerns may partly account for less attention being directed to the final N_2 product of denitrification (Davidson and Seitzinger, 2006). The last step of denitrification is also critically important, because it is a permanent sink for reactive N in the

environment. However, some studies use the parameter 'excess N_2' (i.e. the N_2 concentration above that expected from equilibration with the atmosphere) to quantify denitrification (Wilson et al., 1990). 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^{-1} removal, respectively. Excess N_2 production is the termination of denitrification pathway and is the permanent sink of NO_3^- without any environmental concern. Therefore, it is now increasingly interesting research for the better understanding of the N cycle and N budget in varying environmental conditions.

2.5.9 By-products of the transformation processes

The production of by-products depends on the availability of electron donors and the existing environmental conditions. The fate of the oxygen rejected at each step of the denitrification process depends on the electron donors present (Buss et al., 2005). Where organic carbon acts as the electron donor, the oxygen forms bicarbonate ions and CO_2 but if a sulphide mineral is the electron donor, sulphate ions are formed (Thyalakumaran et al., 2008). Under potent anaerobic conditions, CO_2 can be reduced to CH_4. When sulphide or sulphur containing minerals/metal bound sulphur acts as electron donors, reduced Mn or Fe can be released. Neutral and basic conditions favour the release of N_2 rather than N_2O.

2.6 Environmental conditions controlling denitrification

2.6.1 Introduction

Factors influencing the denitrification and other NO_3^- removal processes are, e.g. distribution of N-sources to soils and groundwater, hydrochemical characteristics such as the distribution and availability of reactive substances maintaining denitrification reactions, and physical properties like sediment heterogeneity, i.e. distribution of hydraulic conductivities, pore space and density of the drainage network. These factors, being spatially and temporally heterogeneous, can be used as the predictors of field scale denitrification rates.

2.6.2 Electron acceptors in groundwater

Microbial denitrifiers in groundwater obtain energy from the oxidation of organic compounds (organic C) or inorganic compounds e.g. reduced iron (Fe), manganese (Mn, III) and sulphur (S). Organic carbon tends to be oxidized preferentially with the electron acceptor that supplies most energy to the microorganisms, namely free oxygen (O_2). Once oxygen is consumed, facultative anaerobes (bacteria capable of growing with or without oxygen) use nitrate as an electron acceptor. As NO_3^- becomes depleted, reduction reactions generally proceed through manganese and iron oxides, then sulphate, and then hydrogen and CO_2, until finally generating CH_4 (Rivett et al., 2008). This redox reaction sequence is commonly seen along groundwater flow lines in aquifers (Seitzinger et al., 2006; Edmunds et al., 1982) and in landfill leachate plumes (Christensen et al., 2000). This redox sequence can also be developed across groundwater recharge to discharge, and depends on the residence time in aquifer and riparian zones. 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).

2.6.3 Electron donors in denitrification processes

2.6.3.1 Availability of organic C

Denitrifiers need a source of energy to fuel denitrification process, typically C, but other sources may also serve as electron donors. Wallenstein et al. (2006) summarized that C availability can affect the denitrifier community and their expressions. Carbon acts as an energy source as well as decreasing the oxygen level in groundwater, eventually creating an anaerobic environment for denitrifiers. Subsoil denitrification is mainly driven by leaching of organic C from the topsoil and denitrification can be strongly limited according to studies by Brye et al. (2001). Lack of organic carbon to provide energy to heterotrophic micro-organisms is usually identified as the major factor limiting denitrification rates in aquifers (Devito et al., 2000; Pabich et al., 2001). Denitrification in groundwater may be related to the DOC that is carried into the saturated zone with NO_3^- (Seitzinger et al., 2006). The DOC levels in most aquifers are relatively low, typically <5 mg L^{-1} DOC

(Rivett et al., 2007). Cannavo et al. (2004) related denitrification activity to DOC concentration as a limiting factor in groundwater. Based on the stoichiometry for the denitrification process relating NO_3^- and organic matter reaction, Jørgensen et al. (2004) used the following reaction:

$$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O \qquad \text{(Eqn. 2.12)}$$

This stoichiometric reaction indicates that 1mg C L^{-1} of DOC is capable of converting 0.93 mg N L^{-1} of NO_3^- to N_2 (Jorgensen et al., 2004). 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) noted that DOC leached from some agricultural soils contributes negligibly to the denitrification process because DOC in the soils appeared not to be bioavailable. However, the contribution of DOC and other C forms to denitrification are still in debate. Well et al. (2001) summarised that total C can show strongly positive correlated with denitrification. Plant roots exude small organic molecules including sugars, amino acids, organic acids and amides (Neff and Asner, 2001). Kaiser et al. (2002) separated DOC into two categories: low-molecular weight compounds 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. Brettar et al. (2002) reported a positive correlation between denitrification rate and TOC in a soil that contained labile carbon which was assumed to have been relatively bioavailable. Dahl et al. (2007) reported that a f_{oc} (fraction of organic carbon) of 3% in riparian zone sediments was an effective indicator of the potential for denitrification. Recent research to characterize the composition of f_{oc} and its reactivity indicated that sediments containing more oxidized soil organic matter (f_{oc}) are less reactive to DO (Hartog et al., 2004). The geological history of the sediments could be correlated with reduction potential. Sediments that had been exposed to aerobic conditions during deposition and diagenesis yielded soil organic matter with a lower reactivity (Allen-King et al., 2002). These include riparian zones (Puckett, 2004; Puckett and Hughes, 2005; Mayer et al., 2006; Domagalski et al., 2008), hyporheic zones (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). Steventon-Barnes (2002) summarized the DOC for selected UK lithologies where DOC

ranged from 0.62 mg L^{-1} in Lower Cretaceous aquifers to 3.9 mg L^{-1} in Millstone Grit with corresponding f_{oc} concentration of 0.002 and 0.0007, respectively.

2.6.3.2 Organic contaminant C sources

The use of organic pollutants as energy sources for microbial denitrification is not well reported. However, 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 (Rivett et al., 2008), for example, benzene, toluene, ethyl benzene and xylene (BTEX) components from petroleum are often degradable under denitrifying conditions (Johnson et al., 2003; Rabus and Widdel, 1996). 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. Broholm and Arvin (2000) argued that phenol, cresols and related compounds may also be degraded by denitrifying bacteria.

2.6.3.3 Reduced iron

Recent studies have highlighted the effective coupling of Fe and N cycles by employing metallic Fe^0 or FeO(s) (Rakshit et al., 2005). Reduction of NO_3^- by Fe^{2+} can be either abiotic, biotic, or both. The abiotic reduction process is not well understood (Buss et al., 2005). 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. Biological NO_3^- reduction can occur with reduced Fe where organic C is not available, called autotrophic denitrification (Chen and MacQuarrie, 2004). Kamolpornwijit et al. (2004) have successfully reduced NO_3^- concentration in groundwater using Fe^0 as a reactive barrier. There are some evidences that groundwaters containing Fe^{2+} normally contain little or no NO_3^- (Korom, 1992). However, Fe^{3+} can precipitate as an oxyhydroxide or oxide mineral (Buss et al., 2005). Examples of stoichiometric equations for these reactions are given below, but less complete reactions may have end points anywhere along the reduction pathway (Ottley et al., 1997):

$12Fe^{2+} + NO_3^- + 13H_2O \rightarrow 4Fe_3O_4 + NH_4^+ + 22H^+$ (Eqn. 2.13)

$10Fe^{2+} + 2NO_3^- + 14H_2O \rightarrow 10FeOOH + N_2 + 18H^+$ (Eqn. 2.14)

$15Fe^{2+} + NO_3^- + 13H_2O \rightarrow 5Fe_3O_4 + N_2 + 28H^+$ (Eqn. 2.15)

Iron can contribute to both biotic and abiotic denitrification processes. Denitrification can occur in the absence of organic C with reduced inorganic species such as ferrous iron, which is referred to as autotrophic denitrification (Chen and MacQuarrie, 2004; Thayalakumaran et al., 2008). The biotic process of NO_3^- reduction by Fe^{2+} can be due to the common bacterium *Gallionella ferruginea*. This is known to reduce NO_3^- to NO_2^- autotrophically in reduced iron environments (Korom, 1992); the nitrite produced can then be reduced abiotically. 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 (Buss et al., 2005).

2.6.3.4 Reduced sulphur

Reduced sulphur can provide electrons for denitrification, termed as autotrophic denitrification (Thayalakumaran et al., 2008). 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-}). Thayalakumaran et al. (2008) measured low NO_3^- concentrations in groundwater coupled with high ferrous concentrations which evidenced that denitrification reduced nitrate with pyrite as an electron donor. 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):

$$5FeS_2 + 14NO_3^- + 4H^+ \rightarrow 7N_2 + 10SO_4^{2-} + 5Fe^+ + 2H_2O \qquad \text{(Eqn. 2.16)}$$

Thayalakumaran et al. (2008) reported high ferrous iron concentration in 55% of the bores they studied which have low DO concentrations (<2 mg L^{-1}). A wide range of autotrophs and heterotrophs including *Thiobacillus denitrificans* (an archetypal organism) can mediate this reaction. Oxidation of sulphur, therefore, provides a viable alternative electron donor in C limited systems (Moncaster et al., 2000; Tesoriero et al., 2000; Thayalakumaran et al., 2008; Broers, 2004). Kölle et al. (1985) approximated that a decrease of 10 mg N L^{-1} can lead to an increase of approximately 50 mg SO_4^{2-}. However, sulphate reducing environment in aquifer can reduce sulphate concentrations and increase sulphide concentrations (Beller et al., 2004).

2.6.3.5 Availability of multiple electron donors

Multiple electron donors can be available in a single location such as organic C, and metal bound sulphur. Denitrification reactions at some sites may be driven by multiple electron donors, for example, where organic carbon, sulphide and iron minerals are all available Buss et al., 2005). Denitrification process requires an abundant supply of electron donors and suitable geochemical conditions where organic C can support heterotrophic denitrification, and reduced iron and sulphur can support autotrophic denitrification in the same aquifer (Chen and MacQuarrie, 2004) (Figure 2.5). At its simplest, this may occur from a change in lithology along a flow line; for example, Böhlke et al. (2002) identified three different environments of denitrification within a superficial sand aquifer. 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. Thayalakumaran et al. (2008) in lower Burdekin coastal floodplain and Weymann et al. (2008) in sand and gravel aquifer in North Germany found significant nitrate removal via groundwater denitrification being carried out by DOC and pyrite.

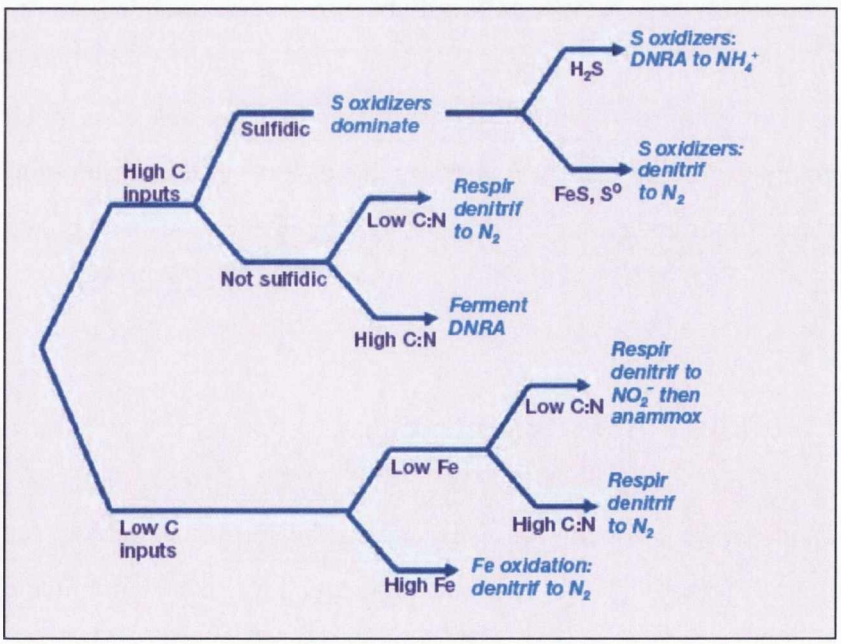


Figure 2.5 Hypothesized controls on predominant dissimilatory pathways of NO_3^- removal. Respir = respiratory; denitrif = denitrification; DNRA = dissimilatory nitrate reduction to ammonium; anammox = anaerobic ammonium oxidation; ferment = fermentative (after Burgin and Hamilton, 2007).

This flow chart summarizes the conditions under which we would expect a particular NO_3^- removal pathway to be important. C inputs refer to labile organic carbon available to microbes. Sulfidic refers to the presence of significant amounts of either free sulfide (H_2S or S^{2-}), elemental S (S^0), or metal-bound sulfides such as FeS_2, all of which tend to be abundant in sediment environments with moderate to high sulfate in overlying water and high labile C inputs to support microbial sulfate reduction. Of these S forms, only free sulfide inhibits denitrification and thus promotes DNRA. The C: N ratios refer to the ratio of labile organic carbon to NO_3^-.

2.6.4 Oxygen concentrations in groundwater

Denitrifying microbes are essentially facultative anaerobes, even though aerobic denitrification also likely to occur (Cannavo et al., 2004). Several authors identified the DO concentration under which denitrification can potentially occur, for example 2-3 mg L^{-1} (Bates and Spalding, 2005) and 4 mg L^{-1} (Böhlke and Denver, 1995) in agricultural fertilizer plume. The denitrification process is thermodynamically less favourable than the reduction of DO (Rivett et al., 2008). While a water sample from a piezometer may be measured in tens or hundreds of millilitres, the amount of water surrounding a 1 mm diameter microbe will be measured in the scale of 10^{-9} ml (Rivett et al., 2008). Therefore, only a relatively very small volume of water, isolated from mixing with the bulk oxygenated groundwater needed within which denitrifying bacteria can begin to respire nitrate. In studies in which aerobic denitrification has been postulated, denitrification is 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, 2005).

2.6.5 Inherent NO_3^- concentrations

Substrate nitrate concentration is one of the important factors that control denitrification process. Increased NO_3^- concentration can increase denitrification where denitrification potential is high, but generally increased NO_3^- affect mole fraction rather than total denitrification. Excess NO_3^- 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). Magalhaes 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^{-1}, coupled with a decrease in the denitrification efficiency. Smith et al. (1988) and Korom et al. (2005) have reported that the kinetics of denitrification at concentrations >1 mg N L^{-1} are zero order (i.e. independent of concentration), suggesting that supply of electron donors controls the rate. Therefore, high substrate NO_3^- concentrations increases the N_2O: N_2 ratio by accumulating N_2O and inhibiting its reduction further to N_2.

2.6.6 Effects of pH on denitrification

The impact of pH on denitrification in soil is extensively reported. The pH range preferred by heterotrophic denitrifiers is generally between 5.5 and 8.0 (Rust et al., 2000). The 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. The pH of the groundwater is mostly neutral to slightly alkaline with no obvious spatial and temporal patterns (Thyalakumaran et al., 2008). The rate of autotrophic denitrification by reaction with Fe^{2+} is also pH controlled (Buss et al., 2005). Strongly acidic environments (pH < 5) inhibit denitrification and tend to arrest the denitrification chain with the formation of NO_2^- 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). Rust et al. (2000) quoted an acceptable upper limit for pH of 8.3, above which denitrification is arrested. Denitrification can again increase pH by producing CO_2 and OH^-, which when combinedly, can release HCO_3^-. However, this can buffer groundwater to keep pH around neutral.

2.6.7 Temperature

Reaction rates are typically assumed to double for every 10°C increase in 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). Groundwater temperatures are typically around 10°C, with the exception of shallow groundwaters impacted by extreme surface temperatures. Lind (1983) confirmed that denitrification in subsoil at in situ (10°C) temperatures was significantly slower than at laboratory temperatures (25°C). However, Tsushima et al. (2002) found that groundwater temperatures 11.8-13.9°C would not be particularly low as most of the denitrifying

microorganisms would be reasonably active at these temperatures. 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 was observed down to 2°C; between 2 and 5°C, rates were approximately 5 mg N L^{-1} d^{-1} and; between 10 and 20°C, rates increased to 15–30 mg N L^{-1} d^{-1}. Carrera et al. (2003) measured denitrification rates at a wide range of temperatures with lowest denitrification rates at 6°C and highest at 25°C. 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.

2.6.8 Microbial communities

The general requirements for denitrification to occur are microbial denitrifiers, available NO_3^- (electron acceptor), energy source (organic C, reduced Fe, Mn and S or molecular H) and an anaerobic condition. The abundance of denitrifying community is generally assumed to be ubiquitous and the denitrifying genes are reported to be widespread in phylogenetically distant organisms (Linne von Berg and Bothe, 1992), in surface water, soil and groundwater (Beauchamp et al., 1989), 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), but their expressions required favourable environmental conditions.

2.6.9 Availability of micronutrients

Microbes involved in all kind of denitrification processes (biotic/abiotic) obtain their energy for metabolism and growth from the oxidation of organic carbon, sulphide minerals or reduced iron and manganese compounds. Their metabolic requirements for N can be met by available NH_4^+ or organic N in the environment, or from the direct assimilation of NO_3^-. Bacteria require carbon, phosphorus, sulphur and micro-nutrients and heavy metals (such as B, Cu, Fe, Mn, Mo, Zn and Co) for effective metabolism (Buss et al., 2005). Phosphorus availability might be expected to be a key limiting factor in aquifer systems due to its often reduced mobility relative to NO_3^-. 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) and field-observed septic-system wastewater plumes (Spiteria et al., 2007).

2.6.10 Salinity

Salinity is linked to the EC and pH, and thus affects microbial community structure dynamics and activities as well. Specific conductivity is a measurement of sample's ability to conduct electric current. Dissolved gas (e.g., N_2O and N_2 produced via denitrification) solubility is significantly affected by salinity. High salinity is known to inhibit, but not necessarily completely arrest, denitrification (Rivett et al., 2008). Relatively few studies are available; nevertheless, Dincer and Kargi (1999) showed that denitrification was inhibited by concentrations of salt greater than 20 g L^{-1} sodium chloride. Usisikh and Henze (2004) found that denitrification rates were reduced to 10% of the maximum when Cl^- concentrations were between 5 and 97 g L^{-1} Cl^-. In estuarine and marine environments, however, denitrification rates do not appear to be affected by the salinity in which they occur (Magalhaes et al., 2003).

2.6.11 Hydrogeological factors

2.6.11.1 Permeability and residence time

Past research has demonstrated that nitrate removal is dependent on hydrogeologic settings and soil characteristics, which affect groundwater flow path chemistry (Vidon and Hill, 2004). Soil particle size distribution affects the biogeochemical processes to occur in soil. Fine particles provide high surface area to volume ratio for microbial growth (Blakey and Towler, 1988). The exception is when pore spaces are too small to permit microbial growth. 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 0.7μm (Whitelaw and Edwards, 1980; Rees, 1981). In addition, biotic activity appeared to be restricted to the fissure wall vicinity (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 mm) was sufficient for biofilms to form and high flow velocities within the fractures actually tended to inhibit biofilm growth due

to shear stresses. Soil drainage class strongly influences N cycling in agricultural, forest and riparian soils (Mosier et al., 2002). Generally, NO_3^- leaching increases with the increase in soil permeability, whereas denitrification decreases. Tsushima et al. (2002) suggested that groundwater with low DO had an over five fold longer residence time than that in the other area. There is little catchment based research on NO_3^- delivery to groundwater in uplands and riparian areas, while the natural NO_3^- dynamics in groundwater along its flow path is also unknown.

2.6.11.2 Groundwater table fluctuations

Groundwater table fluctuations reflect the pattern of rainwater recharge and drainage to and from groundwater which has significant implication on groundwater hydrochemistry. It shows the change in the depth of unsaturated zone overlying the saturated zone over the sampling period. The GWT is known to play a regulatory role in the functioning of shallow groundwater ecosystems by supplying organic matter for heterotrophic metabolism (Baker et al., 2004). Groundwater table fluctuations can affect denitrification and N_2O mole fractions due to its connection to hydrogeochemistry. Young and Briggs (2007) suggested that GWT has a strong influence on the fate of NO_3^-, presumably from constraints on redox conditions and associated impacts on denitrification. However, GWT can influence the extent of denitrification pathway (NO_3^- to N_2), resulting fractionation of its end products. It was obvious that N_2O production was variable with time giving higher concentrations during high water recharge and low groundwater temperatures which might increase the DO concentration in groundwater (Deurer et al., 2008). Different amounts of groundwater recharge resulting in the fluctuations in water table as well as in the depth of saturated zone could be a reason for the variability of N_2O concentrations over time (Von der Heide et al., 2009). The authors also reported a strong negative correlation between GWT fall and N_2O flux. They observed temporal variations in groundwater N_2O emissions, being with lower in October (0.329 mg L^{-1}) and higher in May (0.611 mg L^{-1}). Water recharge could also increase leaching of groundwater DOC and NO_3^--N concentration, and hence increase heterotrophic denitrification resulting in an accumulation of N_2O (Davidson, et al., 1993). Rasiah et al. (2003) found that in a fluctuating GWT, NO_3^- concentrations increased with increasing water table height. Groffman and Tiedje (1988) concluded form their denitrification study that water movement by capillary rise to the upper soil horizons allows the maintenance of both aerobic and anaerobic conditions for nitrification and

denitrification to occur. Geological control over denitrification was also demonstrated in two Maryland catchments studied by Böhlke and Denver (1995). 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 (Starr and Gillham, 1993). Pabich et al. (2001) found that for a sand and gravel aquifer, with an unsaturated zone thickness of less than 1.25 m, DOC concentrations exceeded 20 mg L^{-1}, while with an unsaturated zone thickness of greater than 5 m, DOC concentrations never exceeded 2 mg L^{-1}. Therefore, extensive research on GWT and underlying N dynamics can give more insights about the associated risk of nitrate delivery to groundwater and indirect N_2O emissions to the atmosphere.

2.6.12 Inhibitory substances

Denitrification can be inhibited by the presence of heavy metals, pesticides and pesticide derivatives (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 (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 Baath (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).

2.7 Physical transport processes of NO_3^- in subsoil and groundwater

2.7.1 Nitrogen leaching from soil

Organic N in the soil is mineralised to ammonium under appropriate environmental conditions (aerobic conditions), which is quickly nitrified to NO_3^- and then becomes available for plant uptake and leaching. Ploughing enhances nitrification and increased leaching loss of NO_3^- in arable soils. Conversely, NH_4^+ can be fixed at the exposed surface of clay minerals that have three layer lattice structures e.g. vermiculite, illite or montmorillonite. This happens because hexagonal spaces with similar diameters to NH_4^+ and K^+ are opened to adsorption of positively charged species. Soil and sediment's capacity to retain NH_4^+ is variable. High K^+ concentration is a limiting factor to fix NH_4^+,

whereas high NH_4^+ concentration combined with drying conditions tend to increase NH_4^+ fixation with clay (Whitehead, 1995). Average NO_3^- leaching from terrestrial ecosystems in Central Europe is 15 kg ha^{-1} y^{-1} (Werner, 1994). Soil texture and type affect NO_3^- leaching rates, with coarse permeable soils allowing more leaching through larger, better connected pore spaces (Goss et al., 1998). Light or medium textured soils with rapid infiltration and hydraulic conductivity are more vulnerable to NO_3^- leaching than poorly drained soils (Ryan et al., 2001). The definition of groundwater vulnerability (NRA, 1995) and the designation of NO_3^- vulnerable zones (Defra, 2002) recognize that sandy soils lead to higher NO_3^- leaching to groundwater than clayey soils (Buss et al., 2005). Ploughing can release the N retained in clay soils due to its exposure to oxygen. Macropores (such as root holes, worm holes and desiccation cracks) may facilitate bypass flow around the shallow root zone area of most active denitrification. Zhu et al. (2011) measured about 75% of total N lost via base flow from a mixed land use system, of which 58% was NO_3^--N. Therefore, NO_3^- leaching from below rooting zone to groundwater varies with the biogeochemical processes in unsaturated zone.

2.7.1.1 Factors controlling nitrate leaching from soils to groundwater

Nitrate is completely soluble in water in the presence of all cations likely to be in the soil solution and it is not adsorbed. It is thus vulnerable to being washed out of the soil by percolating rainfall or irrigation water. Rainfall or irrigation water will infiltrate vertically downward to groundwater once water saturation exceeds the infiltration capacity. Movement of percolating water through the unsaturated zone is due to gravity and includes transport of soluble negative ions (as clay particle are negative charged) downwards out of the rooting zone as far as the water table. Temperate regions are more favourable for groundwater pollution by NO_3^- due to its climatic conditions. Downward movement of water through the profile can cause NO_3^- to leach, with the magnitude being proportional to the concentration and water flux (Pierzynski et al., 2005). However, in some soils, e.g. acidic soils clay can absorb nitrate which have positive charge. Reactive N forms other than NO_3^-, such as NO_3^-, NH_4^+ and soluble organic N, can be leached out but it depends on their availability. Ammonium can be adsorbed on to clay particles before it can reach the water table, although some can pass through if the exchange capacity of clay is exceeded. Nitrite is a very unstable form of N that quickly transforms to NO_3^- or to NO and N_2O or to N_2 processes which depend on the prevailing environmental conditions. Therefore, NO_2^- concentrations in soil water are generally low and the risk of NO_2^- pollution of

groundwater is very small. Dissolved Organic N (DON) can be leached out mainly in free draining conditions or if fracture/ cracks occur vertically across unsaturated zone leading to quick transport, resulting in low residence time in which to be mineralized. Two fundamental parameters determine the amount of NO_3^- leached from a plant rooting zone to groundwater are the volume of water draining out and the amount of accumulated NO_3^-, that is, NO_3^- surplus to the plant and microbial requirements (Di and Cameron, 2002). Climatic conditions like rainfall, temperature and soil moisture conditions are important determinants for water draining below the root zone. They also influence the microbial and chemical processes in soil. Soil physical conditions like texture, porosity, structure, organic C, C: N ratio control NO_3^- residence time and biogeochemical processes. Light soils are more vulnerable due to their draining conditions. Farming systems and practices can affect the potential for NO_3^- leaching due the amount and timing of inputs and the nature of crops. At the end of the growing season and before the beginning of a growing season, NO_3^- leaching can be higher than during the growing seasons. Di and Cameron (2002) ranked NO_3^- leaching in order of increasing significance as: cut grassland < grazed pasture or arable cropping < ploughing of pasture < market gardens.

2.7.2 Nitrate transport in unsaturated soils

According to Tesoriero and Voss (1997), a proper prediction of NO_3^- leaching in the unsaturated zone is essential for the assessment of groundwater contamination and ultimately for development of a nutrient management protocol. Usually, nitrification occurs mostly in the aerobic unsaturated zone, and it is thought generally that it is an oxic process (Joekar-Niasar and Ataie-Ashtiani, 2009). However, NO_3^- is soluble in water, and is not significantly adsorbed by clay-rich soils, since it is an anion. As a non-sorbing solute, NO_3^- typically moves at the same velocity as the water in which it is dissolved. Piston flow or piston displacement happens when a soil is at field capacity and receives more water than is utilized for evapotranspiration, so that no more water can be retained and the surplus moves downwards. However, in soil NO_3^- follows a reactive transport therefore, it transforms to NO_2^- and N_2O to N_2. Pressure changes within the soil air space in unsaturated zone may occur due to temperature, barometric pressure, wind and infiltrating water effects (Clough et al., 2005). The movement of NO_3^- and its transformation products are therefore subject to conversion to the stable end product (N_2). 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 the Richards Equation in the unsaturated zone (Fetter, 1999). This process mainly depends on the amount of water content in the unsaturated zone. With decreasing water saturation, hydraulic conductivity values decrease proportionately more than effective porosity (Van Genuchten, 1980). The ammonium ion can be immobilized geochemically by adsorption to the soil or rapidly oxidized to NO_3^- (Joekar-Niasar and Ataie-Ashtiani, 2009). Clough et al. (2005) concluded that the movement of NO_3^- depends of the amount of infiltrating water, entrapped air pressure, groundwater table fluctuation and ebullition of gases. The authors (Clough et al., 2005) also showed that N_2O transforms to N_2 while it diffuses upwardly from deep soil layers. The N cycle in soil and subsoils are complex (Jarvis, 1998) and studies therefore follow an integrated approach to include the losses via other processes that are linked to, or interacting with leaching (Jarvis, 2000).

2.7.3 Nitrate transport in groundwater

Physical NO_3^- transport processes in groundwater may include advection, hydrodynamic dispersion and diffusion. As NO_3^- is transported downstream, it is subject to retention via biotic and abiotic processes (Royer et al., 2004). In many cases, the study of nitrogen transport is complicated by the presence of various N species and the transformations that can occur in the saturated zone due to ambient microbial processes (Lee et al., 2006). Advective transport dominates the transport of solutes in aquifers (Buss et al., 2005). Diffusion occurs as a result 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 (Buss et al., 2005). Tsushima et al. (2002) suggested that retention by advection in floodplain groundwater is negligible. In fractured rocks, the fluids travel faster through the fractures than through the matrix, causing *dispersion* over a larger scale (Buss et al., 2005). 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). In Irish limestone aquifers, porosity is virtually confined to the fractures. Fractures 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). Daly (1995) commented that fracture in Irish subsoils are limited to 3 m bgl and not very common. Gerke and Van Genuchten (1993) observed

dual porosity effects in even seemingly homogeneous coarse-grained materials. Jørgensen et al. (2004) showed that at low flow rates in a macroporous (fractured) till, NO_3^- is retarded relative to a bromide tracer. Bromide can be retarded by matrix diffusion relative to a non-diffusing colloid phase (McCarthy et al., 2002). However dilution can reduce groundwater NO_3^- concentration in shallow GWT (van Beek et al., 2007). Therefore, any NO_3^- transformations in groundwater zones are required in order to understand and evaluate its transport and attenuation from sources to receptors.

2.7.4 Physical NO_3^- retention

Sorption of NO_3^- 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 some soils. Clay et al. (2004) showed that NO_3^- was retarded relative to bromide in a smectitic clay-loam soil. There appears to be no information in literature that has reported NO_3^- sorption. Nitrate adsorption to solid phases can be important mechanism of NO_3^- removal from waste water (Chabani et al., 2007). Davidson et al. (2003) hypothesised that the NO_3^- reacts with the aromatic ring structures of dissolved organic matter to produce dissolved organic N compounds. These may then be adsorbed to soil or be taken up by plants and bacteria. However, estimating NO_3^- retention requires a good knowledge of the hydrogeological settings of the aquifers.

2.8 Biological pathways of NO_3^- depletion in subsoil and groundwater

2.8.1 Background

Biological removal of nitrate from groundwater passing through or over sediments is assumed to be primarily due to respiratory denitrification by bacteria, producing gaseous N_2O or N_2 as a byproduct of organic matter oxidation. Another pathway is assimilation into algal or microbial biomass, producing organic N that may be remineralized later. Heterotrophic respiration of organic matter can be either aerobic or anaerobic. The process of respiratory denitrification is a form of anaerobic respiration in which NO_3^- serves as an alternate electron acceptor. Some common electron acceptors are ranked in order from highest to lowest efficiency of energy yield; $O_2 > NO_3^- > Fe^{3+} > SO_4^{2-}$. In respiratory denitrification, nitrate acts as the terminal electron acceptor for the oxidation of organic

matter under anaerobic conditions; in aquatic sediments, most of the NO_3^- is usually converted to N_2, with a variable but small fraction escaping as nitrous oxide (Figure 2.6). Denitrification rates have been estimated in soils, wetlands, groundwater in floodplains, and surface waters, but estimates vary greatly within and among environments, as well as between different measurement techniques.

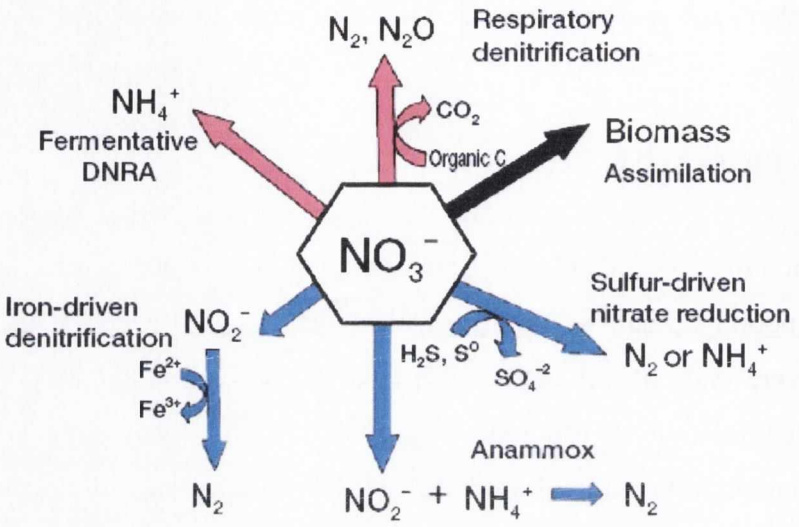


Figure 2.6 A conceptual diagrams of the different possible nitrate removal pathways. Blue arrows denote autotrophic pathways, while purple arrows denote heterotrophic pathways (after Burgin and Hamilton, 2007)

However, while NO_3^- disappearance in soils and aquatic sediments is usually assumed to be largely due to denitrification. This discrepancy between local denitrification estimates and the large losses of NO_3^- at the landscape scale remains difficult to reconcile (Burgin and Hamilton, 2007). One possible explanation can be that adequate methods have not yet been designed to extrapolate from local, site-specific rates to entire ecosystems. An alternative explanation is that much of the NO_3^- removal can be attributed to processes other than respiratory denitrification or assimilation (Burgin and Hamilton, 2007; Rütting et al., 2011). New research has pointed to the importance of processes that remove NO_3^- in freshwater ecosystems, including DNRA (Tiedje, 1988), anammox (Jetten, 2001), denitrification coupled to sulphide oxidation (Brunet and Garcia-Gil 1996; Otte et al., 1999), and reduction of NO_3^- coupled to abiotic or biotically mediated oxidation of iron (Davidson et al., 2003; Weber et al., 2006).

2.8.2 Denitrification in diverse hydrogeochemical conditions

2.8.2.1 Denitrification in subsoils

Nitrate transformation within the root zone is well documented (Ibendahl and Fleming, 2007), but its movement and transformation in prevailing geochemical conditions below the rooting zone are less well understood (Jarvis and Hatch, 1994). Denitrification in subsoils has been suggested as an important process for excess NO_3^- removal, accumulated in soil profile, before leaching to groundwater or discharging to surface waters. The thickness of unsaturated zone varies from several meters in different regions and composed of different types of parent materials. Past researchers have found microbial 'hot spots' with significant denitrification activity in patches of organic rich subsoils at depths of several meters (Hill et al., 2004) and in urine treated subsoils (Dixon et al., 2010). Denitrification in subsoils is controlled by the hydrologic regimes, anaerobiocity, sufficient supply of energy source e.g., organic C and availability of nitrate because denitrifiers are generally reported to be available. For example, hydric subsoils in riparian zones serves as a useful indicator of groundwater nitrate removal capacity in stratified deposits (Groffman et al., 1996; Gold et al., 2001). Considerable losses of N through denitrification in subsoils in peat lands were measured by van Beek et al. (2004). Highest N_2O emissions in subsoils were measured when GWT was at 40 cm bgl, whereas lowest were measured when GWT was at both 5 and 40 cm bgl (Jungkunst et al., 2008).

Subsoil can be composed of homogeneous materials such as alluvial deposits or complex heterogeneous materials such as glacial till. The nature of the stratification of deposits in subsoil influences the solute and gaseous transport through subsoils. In a homogeneous porous medium, the solute is dispersed via a tortuous path through partially saturated pores, whereas in a fractured porous medium, the transport process is more complex (Buss et al., 2005). Kellogg et al. (2005) suggested that abrupt changes in C accumulation occur in glacial till, but in alluvial deposits C accumulation is gradual or similar. Sources of C in subsoils are generally the decomposing vegetations preferentially passed below the rooting zone via root channels or fractures, patchy distributions of animal excreta and organic manures applied in the land. 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. 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). Cannavo et al. (2004) studied the 1.6 m thick unsaturated zone of

calcareous organic silty clay to assess the amount of denitrification beneath a field of decomposing crop residues. Solutes diffuse in and out via the inter-aggregate spaces, or in lower permeability environments, interconnected macropores. 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. Jacinthe et al. (1998) found that small patches of organic matter representing <2.0% of the soil mass in the C horizon of poorly drained riparian soils function as 'hotspots' of denitrification activity. Hill et al. (2000) measured high rates of denitrification in narrow zones near interfaces between aquifer sands and either peats or buried river channel sediments at depths of several meters. Geology of the surface can be another important factor which can control the movement and transformations of solutes in unsaturated zone coupled with the activities of denitrifiers. For example, 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 (Johnson et al., 1998). 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. These contrasting results are indicating that NO_3^- attenuation by denitrification or other pathways in chalk aquifer under Irish agroecosystems are of great interest.

2.8.2.2 Denitrification in groundwater in a limestone aquifer

An investigation into the hydrogeochemistry and the abundances of NO_3^- with regards to denitrification in limestone aquifer might be of interest as this type of aquifer reported to be contaminated with NO_3^-. Limestone does contain appreciable quantities of organic carbon and, in more argillaceous units, pyrite (Bottrell et al., 2000). Wilson et al. (1990) presented evidence for denitrification that includes concentrations of excess N and isotopic ratios. By analogy with sulphate-reducing reactions in the aquifer, Bottrell et al. (2000) concluded that the potential for denitrification in the shallow confined zone is poor. 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. However, denitrification in such an aquifer environment is still extremely difficult to evaluate, and warrants more research. Direct measurement of dissolved N_2O and N_2 can give more realistic insights about the role of denitrification in such aquifers.

2.8.2.3 Denitrification in sandstone dominated aquifer

The sandstone aquifers can exhibit denitrification where they are confined and flow is sufficiently slow for long contact time between the aquifer and water (i.e., high residence time). However, sandstones are very deficient in organic C content. Parker et al. (1991) refer to an unpublished report considering the prospects for denitrification in such aquifers. With a source of dissolved organic carbon from a sewer leakage, pollutant NO_3^- in the Triassic Sherwood Sandstone beneath Nottingham in the UK was readily denitrified (Fukada et al., 2004). Groundwater zones characterised by sandstones aquifer can provide less entrapped air and less oxygen saturation with adequate carbon as electron donor for denitrification to occur (Buss et al., 2005). In Irish sandstone dominated aquifers, no denitrification study was reported so far that needs to be explicitly studied. The measurement of dissolved gases e.g. O_2, N_2, Ar and N_2O can provide accurate estimation of denitrification capacity of groundwater in such aquifer.

2.8.2.4 Denitrification in a shale containing aquifer

Shale might contain adequate amount of potential electron donors e.g., organic carbon and sulphide mineral. McMahon et al. (1999) measured denitrification and reported that denitrification in the shale was a sink for NO_3^- in groundwater. Schultz et al. (1980) reported mean concentrations of organic carbon and sulphide (S^{2-}) in Pierre Shale of 0.94 and 0.25 weight percent, respectively. However, the relatively large potential first-order rate constant for denitrification in the shale indicated that percentage of NO_3^- uptake by the shale could be considerably larger in areas where NO_3^- is transported more rapidly into the shale by advection.

2.8.2.5 Denitrification in aquitard sediments

Denitrification in aquitard sediments is occasionally time very important because many water supplying aquifers are confined by overlying aquitard sediments. Nitrate attenuation in aquitard sediments with increased sulphur content showed evidence of autotrophic denitrification, which eventually increased SO_4^{2-} content in pore water (McMahon, et al., 1996). 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 (McMahon, 2001).

In unweathered aquitards, solute transport is controlled mostly by diffusive processes but advection can sometimes be significant (Robertson et al., 1996). McMahon et al. (1996) suggested that aquitard sediments or glacial tills separate productive aquifers from the near surface NO_3^- contamination. These glacial tills might have different hydrogeological compositions e.g., different concentrations of nitrate. Nitrate depletion in such aquifer can be due to denitrification based on the evidence of N isotopic behaviour and the presence of denitrifying bacteria, even though the rate of depletion is very low (McMahon et al., 1996). In practice, heterotrophic denitrification utilizing organic carbon present in the sediments or in the pore water is also likely to occur.

2.8.3 Denitrification in groundwater–surface water interface

2.8.3.1 Background

Buss et al. (2005) suggested that there is growing interest in the use of riparian zones and wetlands as buffers to surface water bodies from non-point source pollutants such as NO_3^-. This interface between groundwater and surface waters are playing significant role in surface water NO_3^- delivery by enhanced biological and hydrogeological environments. It can be characterised by dynamic zones of horizontal and vertical heterogeneity, where reducing conditions and near-constantly saturated sediments high in labile organic carbon facilitate denitrification (Hill, 1996). These can arise because of their position in the landscape, spatial variabilities in hydrologic regimes, geological heterogeneity, distribution of organic carbon, seasonal influences on temperatures and overall heterogeneity in redox chemistry. The groundwater–surface water interface can be conveniently divided into three zones, which, although they share some general qualities in terms of hydrogeology, hydrochemistry, biodegradation potential and vegetation, also have notable unique qualities (Buss et al., 2005). These are the riparian zone, riparian wetlands and the hyporheic zone.

2.8.3.2 Denitrification in riparian zone, riparian wetland sediments and hyporheic zones

Groundwater-surface water interface is of great environmental and ecological interest due to its unique hydrogeological and biogeochemical nature. Buss et al. (2005) defined this interface with three distinct zones are riparian zone, riparian wetlands and hyporheic zone

(Figure 2.7). The riparian zone is the area adjacent to a stream or river that is dependent on a variably moist regime (Buss et al., 2005). Riparian zones play a key role as a buffer system (Pinay et al., 2007). There is clearly an overlap between riparian zone and shallow permeable aquifer. The key difference between these is the elevation of the GWT during seasonal fluctuations: in a riparian zone, the GWT is expected to reach the soil zone only during the wetter months, whereas in a shallow aquifer the soil would rarely be saturated (Buss et al., 2005). The riparian zone plays an important role in NO_3^- removal before discharging to the surface waters, ranging from 0-100%. Riparian zone has important features of the landscape that can buffer waterways from non-point N pollution (Woodward et al., 2009). Integrating wetlands and riparian buffers into the agricultural landscape has the potential to reduce N losses to surface waters (Kadlec, 2005). However, within the riparian zone the factors that influence the depth of the biologically active zone need to be resolved (Addy et al., 2002).

Another distinct zone of interest in groundwater-surface water interface is the riparian wetlands. Riparian wetland areas are associated with hydrologic regimes where the water table is at or near the surface over the year. Kellogg et al. (2005) defined the riparian wetlands where hydric soils develop due to saturation with shallow water table. Groffman et al. (1996) commented that riparian wetlands can be useful indicator of groundwater nitrate removal. Because, these soils were found to have lower DO, shallower water tables, and higher groundwater nitrate removal rates than nonhydric soils (Kellogg et al., 2005). In riparian wetland areas biogeochemical transitions can occur over very short distances within hydric soils. 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). Aerobic groundwater can become anaerobic within horizontal distances of 5 to 10 m (Nelson et al., 1995). Therefore, the details characterization of hydrologic and biogeochemical properties in riparian wetland is of increasing ecological and environmental interests.

Hyporheic zone is a water-saturated region below and adjacent to a surface water body and the zone in which groundwater and surface waters mix. Curie et al. (2009) attributed NO_3^- depletion in hyporheic zone to denitrification as it occurs when oxygen concentration is below 2 mg L^{-1}, and goes along with a consumption of dissolved organic carbon and a decrease of redox potential. Typically 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, 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. Hancock and Boulton (2005) investigated the impacts of an environmental flow release on water temperature, conductivity, DO, and nitrate concentrations in surface and subsurface (hyporheic) waters at up welling and down welling zones in three sites along the Hunter River, New South Wales, Australia and observed stimulation of hyporheic microbial activity, short time nitrification, and denitrification over time enhancing NO_3^- depletion.

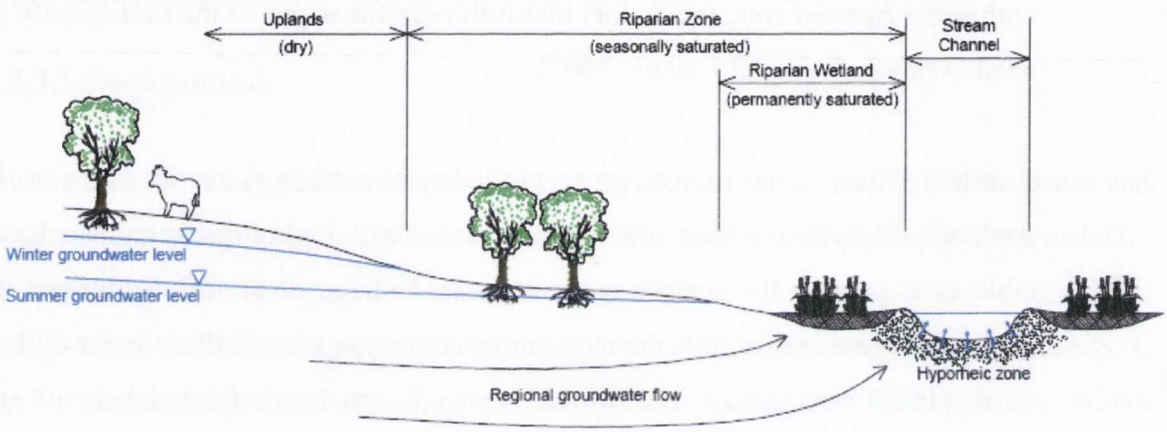


Figure 2.7 Zonation of groundwater wetness within a typical aquifer/river transition zone (Buss et al.,2005)

2.9 Landscape engineering for denitrification enhancement

Land management approaches have been proposed to reduce N losses in agricultural ecosystems such as, improved N use efficiency, managing N inputs and controlled drainage (Dinnes et al., 2002; Jaynes et al., 2004). Application of permeable reactive barriers (PRB) is recently being used successfully to reduce NO_3^- contamination to groundwater and surface waters. The PRB have been shown to be very effective at attenuating NO_3^- in groundwater (EA, 2002). Denitrification in terrestrial ecosystems is enhanced by installing reactive barriers along groundwater flow paths e.g., denitrification walls (Jaynes et al., 2008; Schipper et al., 2010), and denitrification beds (Robertson et al., 2005; Robertson et al., 2009). These are constructed perpendicular to the water flow paths and have comparatively higher hydraulic conductivity (>10 m d^{-1}). Stream bed bioreactors are sometime installed into the existing stream beds or drainage ditches. These engineered

techniques are being successfully carried out to remove substantial quantity of NO_3^- by supplying electron sources e.g., by wood chips, sawdusts etc. Sawdust amended barriers can remove in excess of 95% of nitrate load for at least six to seven years (Robertson et al., 2000). However, pH in the denitrification wall needs to be controlled as denitrification over time can increase pH which can go beyond the suitable range. Therefore, amendment may need to reduce pH by buffering mechanisms with an acid buffer. A horizontal installation constructed with fine grained materials can create permanent anaerobic conditions even when water table goes below it. Application of PRBs may cause clogging with subsequent bypass flow of groundwater contaminants around or through the barrier due to low hydraulic conductivity (Fenton et al., 2008). The installation and construction of denitrification trench are still new and need extensive consideration. They should be designed to intercept as much groundwater as possible (Schipper et al., 2001). The trenches are generally about 1.5 m wide and 1.5 m deep and of variable length. Once excavated the soil is mixed with approximately the same amount of sawdust. More research needs to be undertaken to determine the most effective ratio. The material used should contain labile C sources, and a higher permeability than the surrounding soil to ensure preferential flow of groundwater through the sawdust and soil mixture in the trench (Fahrner, 2002). The key to selecting appropriate bioreactors design depends on the hydrologic conditions and site conditions of the system of interest (Schipper et al., 2010).

2.10 Estimation of NO_3^- mass flux measurement in groundwater

The ability to measure groundwater contaminant flux is increasingly being recognised as crucial in order to prioritise contaminated site cleaning, estimate the efficiency of remediation technologies, measure rates of natural attenuation, and apply proper source terms to model groundwater contaminant transport (Goltz et al., 2007). The risk of NO_3^- contamination and its likely effects can be measured to take remediation measures by quantifying the mass flux of NO_3^-. Mass flux is a measure of the rate contaminant mass is transported, in units of mass per time per area of aquifer orthogonal to the direction of groundwater flow. Einarson and Mackay (2001) argued that contaminant mass flux is more relevant as an indicator of risk at a down gradient water supply well than contaminant concentration in the plume, and would be more useful in helping regulators and remediation decision makers. Contaminant mass flux measurement has been the subject of considerable research in the past 5 years, as scientists, regulators and hazardous waste site

managers have begun to realize the importance of measuring contaminant flux, as opposed to traditional measurements of contaminant concentration (SERDP/ESTCP, 2001). Contaminant mass flux can be a function of aquifer permeability. A smaller source zone in a high permeability region may results in significant contaminant mass flux leaving the area. Various authors applied the mass flux measurement approach to evaluate natural attenuation and quantify natural attenuation rate constants (Bockelmann et al., 2001, 2003; Peter et al., 2004).

2.11 Methodologies for Measuring Denitrification

2.11.1 Direct measurements of denitrification

2.11.1.1 Soil core incubation

Direct techniques mainly use direct measurements of N fluxes to estimate the rate of denitrification. For example, both direct N_2 flux measurements and measurement of N_2O accumulation after inhibition of the $N_2O \rightarrow N_2$ transformation would be direct techniques. The acetylene inhibition technique is based on the inhibition of the transformation of N_2O to N_2 (Sørensen, 1978); the accumulation of N_2O instead of N_2 is readily measured by gas chromatography using an electron capture detector (ECD) and the total N_2O flux is equivalent to the denitrification rate. Acetylene also inhibits nitrification (Hynes and Knowles, 1978), which can be an important source of NO_3^- for denitrification, so this technique can lead to underestimates of in situ rates (Kemp et al., 1990). Several limitations have been identified, including the incomplete blockage of the N_2O transformation to N_2 (Seitzinger et al., 1993), particularly where hydrogen sulphide is present (Koike and Sørensen, 1988). Incubation of intact or repacked soil cores in controlled environments is being widely used to measure N_2O emissions. The direct measurement of denitrification is complicated by a high background concentration of N_2 in atmosphere or dissolved in natural waters. However, recently Scholefield et al. (1997) and Cardenas et al. (2003) developed an automated laboratory incubation system to measure N_2O and N_2 emissions simultaneously and continuously from soil cores. Direct and independent measurement of N_2O and N_2 flux can be carried out by incubating the soils/sediments cores in a temperature controlled environment. The biogenic gases are flushed with an artificial gas mixture (He: O_2 = 15: 85) to remove them from the headspace

of the soil core-containing vessel and then continuous recording of N_2O and N_2 concentrations is carried out using a dedicated gas chromatograph. The system is well equipped with amendment vessels so that different levels of treatments for nutrients and moisture can be possible. Before the amendment of treatment solution soil cores are purged with $He+O_2$ mixture (He: O_2 = 20: 80) to remove the ambient biogenic gases from soil pore space.

2.11.1.2 Natural abundances of ^{15}N

By 2004, more denitrification measurements had been made with isotope pairing than with any other technique in the current decade. Stable isotope composition of NO_3^- is indicative of the source and biological reduction of NO_3^-. However, stable isotopes can be used in various ways for denitrification studies such as H and O in water; N and O in NO_3^-, NH_4^+, NO_2^-, N_2O, and dissolved N_2 gas; S and O in sulphate and sulphide; C in dissolved C; O in dissolved oxygen; C, N and S in organic and inorganic solids (Groffman et al., 2006). The stable isotope for N used the ratios expressed as $d^{15}N$ which compares the fraction of $^{15}N/^{14}N$ of the sample to that of an internationally accepted standard (the air), for example:

$$\delta^{15}N(‰)=\frac{(^{15}N/^{14}N)_{sample}-(^{15}N/^{14}N)_{standard}}{(^{15}N/^{14}N)_{standard}}x1000 \quad \text{(Eqn. 2.17)}$$

where the isotopic ratio reflects molar abundances and 'air' (atmospheric N_2). Sources of N have characteristic isotopic signatures which indicate the specific sources of NO_3^- in the sample. Böhlke and Denver (1995) use $d^{15}N$ with $d^{13}C$, $d^{34}S$, chlorofluorocarbons, tritium and major ion chemistry to determine the application history and fate of NO_3^- contamination in agricultural catchments, 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. The isotopic ratios for O can be given as:

$$\delta^{18}O(‰)=[(^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{VSMOW}-1]x1000 \quad \text{(Eqn. 2.18)}$$

where the isotopic ratio reflects the molar abundances and 'VSMOW' (Vienna Standard Mean Ocean Water). The effect of isotopic fractionation on reactants (r) and products (p) during denitrification is described by Rayleigh distillation equation (Rayleigh, 1896; Clark and Fritz, 1997):

$$(^{15}N/^{14}N)_r = (^{15}N/^{14}N)_{r,ini} * (^{14}N_r/^{14}N_{r,ini})^{\alpha-1} \quad \text{(Eqn. 2.19)}$$

where ini is initial. The fractionation factor a is the instantaneous relation between reactant and product and given as:

$$\alpha = (^{15}N/^{14}N)_p / (^{15}N/^{14}N)_r \quad \text{(Eqn. 2.20)}$$

this can also be used to describe the kinetic fractionation:

$$\alpha = k_r[^{15}N] / k_r[^{14}N] \text{ ; (Eqn. 2.21)}$$

and

$$\varepsilon = (\alpha - 1) x 1000‰ \quad \text{(Eqn. 2.22)}$$

where k_r refers to the first order reaction rate constant. Use of isotopic ratios is becoming a standard technique to identify the occurrence of denitrification. These include studies of denitrification in aquifers (Smith et al., 1991; Widory et al. 2004), riparian zones and the hyporheic zone (Clément et al., 2003; Devito et al., 2000; Mengis et al., 1999), and permeable reactive barriers (Robertson et al., 2000). However, several underlying assumptions are required, namely that added $^{15}N\text{-}NO_3^-$ mixes homogeneously with the $^{14}NO_3^-$ pool, which added NO_3^- does not change the rate of coupled nitrification-denitrification, that isotope fractionation can be neglected, and that diffusion of heavy and light NO_3^- is similar (Middleburg et al., 1996). Experiments using ^{15}N as a tracer for denitrification are made difficult by incomplete labelling of sediment $^{15}NO_3^-$ and $^{15}NH_4^+$ pools. The measurement of $^{14}N^{14}N$, $^{14}N^{15}N$ and $^{15}N^{15}N$ dinitrogen by mass spectrometry is required. Despite concerns about the complete mixing of ^{15}N and ^{14}N isotope pools (Middelburg et al., 1996), this technique generally appears to give reliable estimates of denitrification. Seitzinger (1987) has used a long-term incubation technique to measure direct N_2 fluxes. In this method, overlying water is changed daily with N_2 free water for 10 days to remove most of the N_2 in the system; incubations of about 24-h are then sufficient

to measure N_2 fluxes using gas chromatography. In general, this technique appears to give higher rates of denitrification than the acetylene or ^{15}N techniques (Seitzinger et al., 1993).

2.11.1.3 Isotope tracer technique

Application of ^{15}N enriched nitrate to soil or sediments can be used to directly measure the production rates of N_2O/N_2. Kellogg et al. (2005) and Addy et al. (2002) used an in situ push-pull technique to measure in situ rates of denitrification in the groundwater zones (Figure 2.8) using ^{15}N enriched NO_3^-. They "pushed" (i.e., injected) 10 L of previously collected ground water into well and then "pulled" (i.e., extracted) ground water from the same well after an incubation period. Prior to injection, the ground water was amended with ^{15}N-enriched nitrate and Br^-. Then, this amended solution was adjusted to ambient dissolved oxygen (DO) concentrations to mimic aquifer conditions by bubbling a sulphur hexafluoride (SF_6) gas through the dosing solution. Two conservative tracers, gaseous tracer SF_6 and soluble anion Br^-, provided insight into the recovery of the introduced plume. To minimize the effects of confounding factors such as dilution and dispersion, denitrification rates were estimated from only the "core" of the plume (i.e., the first 2 L of the plume pulled from the mini-piezometer after the incubation period). In sandy media (bulk density = 1.65 g cm^{-3}, porosity = 0.38) the 2 L plume core interacts with 8.7 kg of soil. Results obtained by this method have provided a much better understanding of the regulation of denitrification and interactions with other processes and environmental conditions (Groffman et al., 2006).

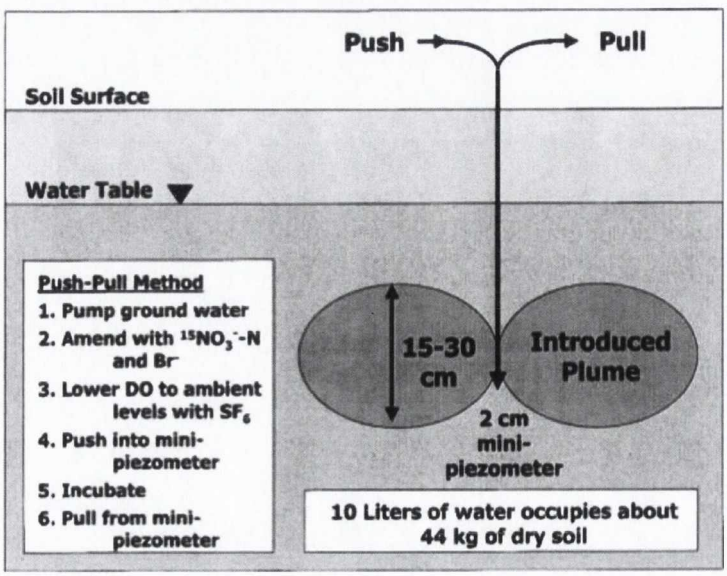


Figure 2.8 Schematic of the in situ push–pull mini-piezometer method (after Addy et al. 2002)

2.11.1.4 Dissolved N_2:Ar ratio analysis

Kana et al. (1994) described a mass spectrometer that is able to rapidly measure N_2 in water at a precision of < 0.05% for the N_2:Ar ratio (Figure 2.9). This made it possible to measure denitrification (specifically net N_2 flux) in sediment core samples using incubations of <12-h, or by measuring the difference in the N_2 concentration between inlet and outlet water of continuous flow incubations. The mass spectrometer utilizes a membrane tube interface that allows dissolved gases to permeate into the vacuum inlet. Practical advantages of the dissolved gas analyser (DGA) over either gas chromatography or conventional mass spectrometers for measurements of N_2 flux include the lack of a degassing step (so that dissolved gases are measured in line with the mass spectrometer), rapid throughput, small sample size, high precision, and simultaneous measurement of O_2 concentration. In addition, N_2 is the direct product of denitrification which avoids errors incurred by measuring proxy products such as N_2O in acetylene inhibition method or ^{15}N in isotope enrichment technique. The continuous flow method is particularly suitable for studying factors that influence denitrification, such as water column nitrate concentration, or process kinetics (Kana et al., 1994, 1998).

Measurement of N_2:Ar ratio enables to measure denitrified N_2 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'. 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^{-1}.

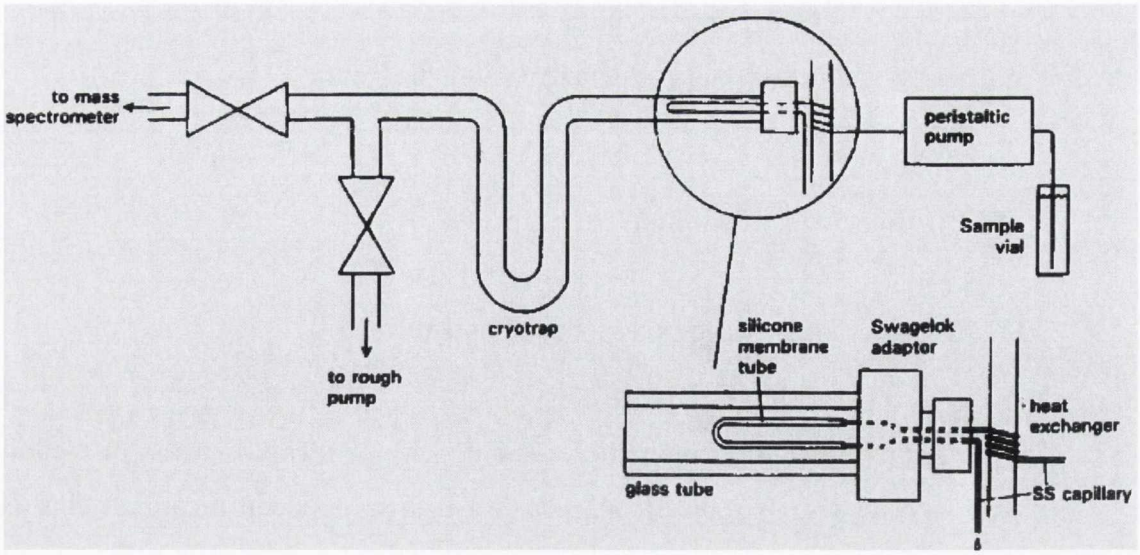


Figure 2.9 Schematic of the sample pumping system and vacuum interface to the quadrupole mass spectrometer (after Kana et al., 1994)

2.11.1.5 Dissolved N_2O analysis

The dissolved N_2O measurement and its mole fraction to total denitrification (N_2+N_2O) can be an important indication of denitrification potential at varying zones/depths of groundwater. Groundwater degassing technique has been widely used for measuring dissolved gases as a measure of denitrification. Hamilton and Ostrom (2007) extracted groundwater dissolved gases at a ratio (water: *He*) of 1:2 (v/v). They collected 30 ml groundwater in a polyethylene syringe and removed 20 ml water with a simultaneous addition of 20 ml *He* and then agitated vigorously for 5 min for equilibration of headspace gas. After equilibration, the headspace gas was immediately transferred into an exetainer. Lemon (1981) collected 250 ml water in a glass bottle and 50 ml (water; bottle headspace = 4:1) was then poured out followed by addition of 1 ml of saturated $HgCl_2$ and the samples were frequently shaken (25°C) for several days. Air for analysis was removed by injecting 10 ml of Hg into a sample bottle, then drawing off an equal volume with a 10 ml gas syringe and injecting it into a 0.5 ml sample loop on a Hewlett-Packard 5730A gas chromatograph with a 63Ni EC detector. Kellogg et al. (2005) analysed groundwater for dissolved gases (N_2, N_2O, ^{15}N, $^{15}N2O$, SF_6) by collecting groundwater using 20 ml syringe attached to a gas-tight stainless steel apparatus and injected into a previously evacuated 150 ml glass bottle capped with a rubber septa. The head space was then filled with high purity helium gas to atmospheric pressure water: *He*= 1:7.5). To sample for dissolved gases, they used the phase equilibration headspace extraction technique, storing samples at

4 °C overnight, shaking and sampling the bottle headspace with a syringe. The dissolved gases concentrations were then calculated using the Henry's Law.

2.11.2 Indirect measurement techniques

2.11.2.1 Stoichiometric process-based estimation

The application of stoichiometric assumptions to measurements of sediment-water exchange provides an indirect approach to estimating denitrification. This calculation requires constant elements ratios (for algae, C: N = 6.6; N: P = 16) associated with the dominant terminal electron accepting processes in eutrophic coastal sediments (Canfield, 1989):

(i) $(CH_2O)\ 106\ (NH_3)16(H_3PO_4)+138O_2 \rightarrow 106CO_2C+16NO_3^-+H_3PO_4+122H_2O$ (Eqn. 2.23)

(ii) $(CH_2O)106(NH_3)16(H_3PO_4)+53SO_4^{2-} \rightarrow 106CO_2+16NH_3+H_3PO_4+53S^{2-}+106H_2O$ (Eqn. 2.24)

Other terminal electron acceptors not represented here (NO_3^- and metal oxides) have been recognized as being important in carbon metabolism in some coastal sediments (Middelburg et al., 1996). The ratios of C: N and N: P shown here are for remineralisation processes, and are often at variance with the bulk ratios observed in sediments (Cornwell et al., 1996). The process of nitrification can change the ratio of C: Oxygen because of the utilization of both N and C by nitrifying bacteria. If the flux of dissolved inorganic N (DIN = $NH_4^+ + NO_2^- + NO_3^-$) from sediments is less than the NH_4^+ remineralisation flux predicted from the measured dissolved inorganic carbon or P fluxes, the difference is assumed to be due to denitrification. In the Giblin et al. (1997) study of benthic fluxes in Boston Harbor, annual DIC: DIN flux ratios ranged from 9.6 to 15.5, considerably higher than those observed for organic matter. This suggests that 31–57% of remineralized N was denitrified.

2.11.2.2 Estimation by 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 NO_3^- concentration caused by mixing of groundwaters with different composition. Neither chloride nor nitrate is affected by chemical processes in groundwater except where NO_3^- may undergo denitrification (Buss et al., 2005) and an increase in the Cl^-/ NO_3^- ratio indicates that NO_3^- removal process e.g., denitrification occurs (Altman and Parizek, 1995; Mengis et al., 1999).

2.11.3 Method for measuring NO_3^- mass flux

Contaminant mass fluxes at the field-scale are usually determined at one or more imaginary control planes running perpendicular to the groundwater flow direction. Several measurement and interpretation methods are available which can be grouped into point-scale and integral approaches. The point-scale approach requires data from a multilevel monitoring network (Bockelmann et al., 2003; Einarson et al., 2000) but the integral approach uses data from a monitoring campaign that has to be conducted at one or more monitoring wells (Bockelmann et al., 2001). Multilevel groundwater monitoring wells can be used in measuring field-scale contaminant mass fluxes (API, 2003). The common method for quantifying mass flux in groundwater is to sample a control plane at a number of multilevel wells each equipped with a number of vertical sampling points (Kübert and Finkel, 2006).

2.12 Conclusion

Denitrification can be an important process in reducing NO_3^- in subsoils and groundwater. Methods for the direct measurement of denitrification are better than the indirect methods due to their potential limitations e.g. nitrification inhibition by acetone underestimates N_2O production. Measurement of dissolved N_2 in groundwater is a precise and rapid method. Application of the isotopic signature in groundwater denitrification is very useful to investigate in situ N cycle processes.

CHAPTER 3. SELECTED SITE CHARACTERIZATIONS AND PIEZOMETER INSTALLATIONS

3.1 Overview of this chapter

This chapter presents the background information on the four agricultural catchments investigated. Among the four sites, a preliminary experiment on subsoil denitrification was conducted in only one site to get insights into the potential of denitrification in a specific soil type and drainage condition. A study plan and type of investigations conducted are shown in section 3.3. The land use at all sites and a simple N balance are presented in section 3.4 and 3.5. The general information on soil type, land management, hydrogeology and geology was gathered through desk study as well as filed sampling.

3.2 Selection of sites based on hydrogeology, land use and climatology

The experimental sites were selected based on the diverse landscape settings and land use with a marked contrast in subsurface hydrogeochemical conditions in Ireland (53°20'N 6°16'W). The Irish climate is cool, humid and maritime, characterized by evenly distributed annual rainfall which varies between 1500 mm along the Atlantic coast to around 750 mm along the south coast. The selected sites were under approximately similar climatic conditions with slightly wetter southwestern part to dryer southeastern regions (Figure 3.1). The mean temperature generally ranged from 4.5° C in winter to 15.5° C in summer. A diverse range of top and subsoil types and drainage conditions, GWT fluctuations, permeability of glacial till and bedrock, and geology of bedrock were considered to carry out the study, and to achieve a concrete and generalized understanding of the abundance of NO_3^- and its reduction via biogeochemical processes.

3.3 Study plan, tests carried out and locations

A study plan including the sites and nature of the tests is presented below in Table 3.1. The study was a mix of hydrology, hydrogeology and biogeochemistry, and conducted both field and laboratory conditions.

Table 3.1 Study type, location and nature of test

Investigation type	Study site	Nature of test
Subsoil denitrification	Johnstown Castle (JC), Co. Wexford	Laboratory incubation of soil core
Hydrology of the study sites	Johnstown castle (JC), Solohead (SH), Oak Park (OP) and Dairy Gold (DG)	Field and desk studies
Hydrochemistry	Johnstown castle (JC), Solohead (SH), Oak Park (OP) and Dairy Gold (DG)	Field sampling and laboratory analysis
Groundwater denitrification	Johnstown castle (JC), Solohead (SH), Oak Park (OP) and Dairy Gold (DG)	Field sampling and laboratory analysis
In situ denitrification in groundwaters	Johnstown castle (JC) and Oak Park (OP)	Filed experiment and lab analysis
Groundwater denitrification underneath cover crop	Oak Park (OP)	Field experiment and laboratory analysis

3.4 Locations and land uses

3.4.1 Johnstown castle (JC; Grazed Grassland)

The JC dairy farm is located at a research farm of Teagasc Environment Research Centre, Co. Wexford (53°20′52″ N 6°15′77″ W). Johnstown Castle Estate occupies approximately 400 ha area, of which almost 200 ha are under woodland, and 8.3 ha are under water (Figure 3.2). About 48 ha area has been under dairy farming systems (grazed grassland) since 1975. Relief in the area is controlled by the underlying bedrock and the constructional form of the glacial deposits. The landform is mostly undulating with slopes rarely exceeding 5°. The farm is located approximately 60 m AOD at the northeastern side to as low as 32 m AOD at the southwest (Figure 3.3). A narrow river, located 50 m to the southwest of the catchment, had its water level approximately at 29.56 m AOD during winter (January 2010).

3.4.2 Solohead Dairy Farm (SH; Grazed Grassland)

The SH dairy farm is located near Limerick Junction in Co. Tipperary (52° 51' N; 08° 21' W). The total area of the farm is 52 ha and has been in dairy farming systems since 1976. The topography of the farm is relatively flat sloping gently ca. 3° from the north-west of

the site. The well SH1A, SH1B and SH1C are located on the top slope and 2A, 2B, 2C, 3A, 3B and 3C are located on the down slope near the drain (Figure 3.4). The land is being used as grazed grassland predominantly composed of perennial ryegrass containing an annual average of 22% white clover in 2009. Because of the supplementation of the grass production with a leguminous grass, clover, the total fertilizer input in this site is comparatively low (Table 3.1). The land is located from 92 to 98 m AOD (Figure 3.3) where the stream water surface in winter (as of January 2010) was at 91.53 m AOD.

3.4.3 Oak Park (OP; Arable land)

Oak Park is an arable land which belongs to the river Barrow catchment in Co. Carlow, southeast Ireland. The OP arable land is on a 10 ha area under spring barley with mustard/regeneration of barley as cover crop rotations located in the National Centre for Arable Crops Research occupying a 225 ha research farm (52° 51' 43" N, 6° 54' 53" W). The land is relatively flat sloping down ca. 2° from north to south direction discharging its drained water to the river Barrow (Figure 3.5). County Carlow has reputations as a tillage county, due to the presence of free draining, light texture soils occupying approximately 65% of its total area (Conry, 2006), whilst tillage systems occupy approximately 22% of the county. The elevation of the land is approximately 54 to 56 m AOD (Figure 3.3) where the GWT in the river Barrow is at 51.59 m AOD as of winter 2010. Cover crop e.g., mustard or regenerated spring barley is being used to reduce nitrate leaching during winter while the land was bare otherwise. The N input in this site as an arable land is lower than the other sites (Table 3.1).

3.4.4 Dairy Gold (Grazed Grassland)

The Dairy Gold dairy farm is known as Moorepark Dairy Gold Research Farm located in the Teagasc Moore Park Research Centre on a 93 ha land (50°07' N, 08°16' W), Co. Cork, which delivers its farm runoff and interflow water to the river Funshion (Figure 3.6). This farm has been under Teagasc since 2002, but it has been under dairy production systems since 1960 managed by Dairy Gold Co-operative Creamery. The land has undulating topography with a slope of approximately ca. 5° and elevation of about 46 to 61 m AOD (Figure 3.3). It is used as an experimental farm under grazed grassland with different

stocking rates and fertilizer N rates ranging from 2.0 to 2.94 stocking rates and 165 to 325 kg ha^{-1} N fertilizer showing the mean total N input of 298 kg ha^{-1} (Table 3.1).

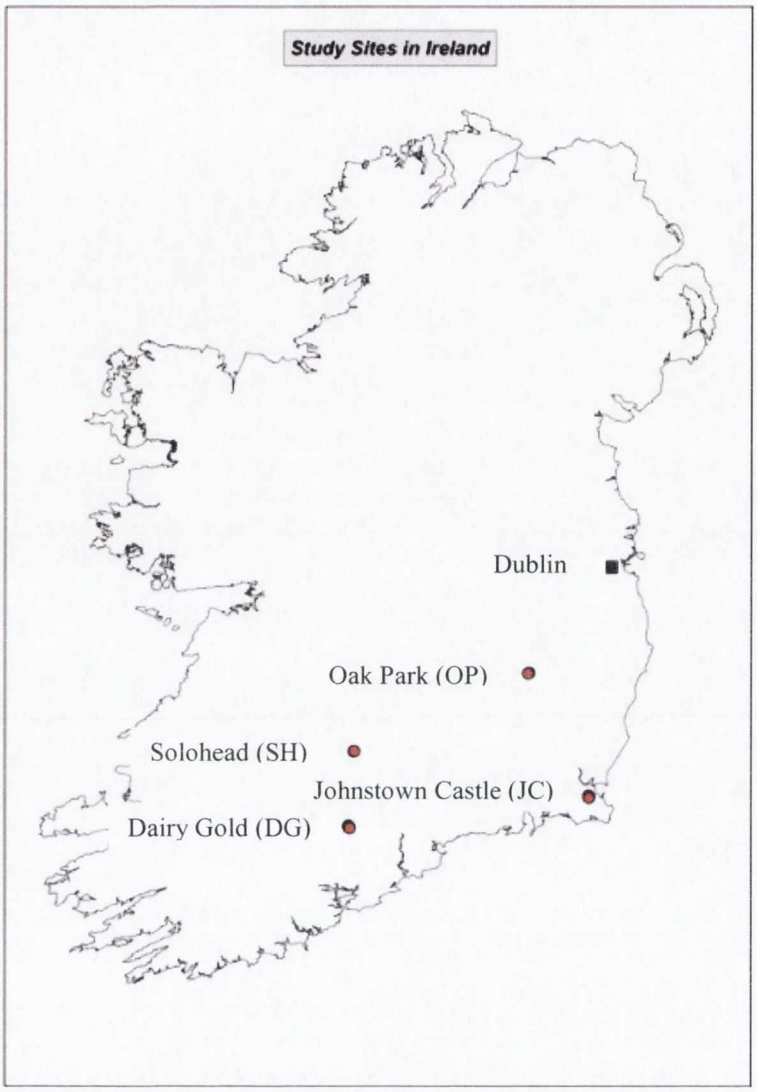


Figure 3.1Locations of study sites in Ireland

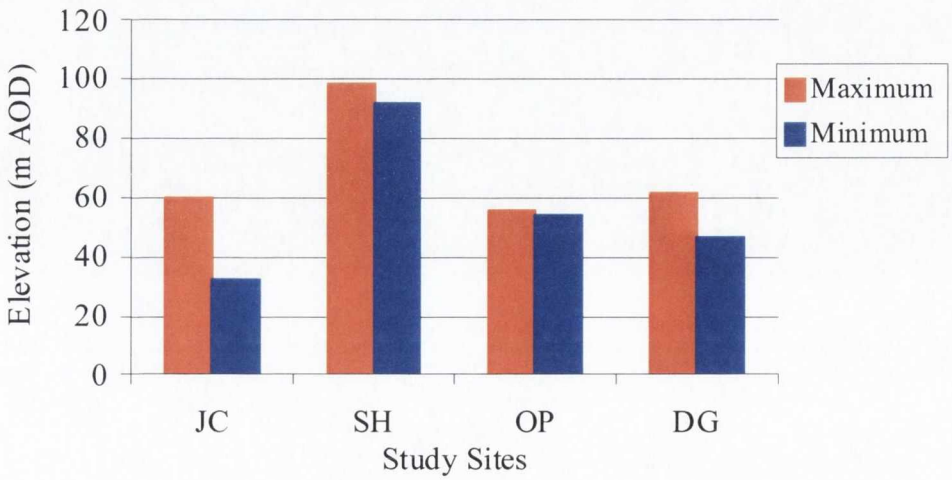


Figure 3.2 Maximum and minimum ground level elevation at each site

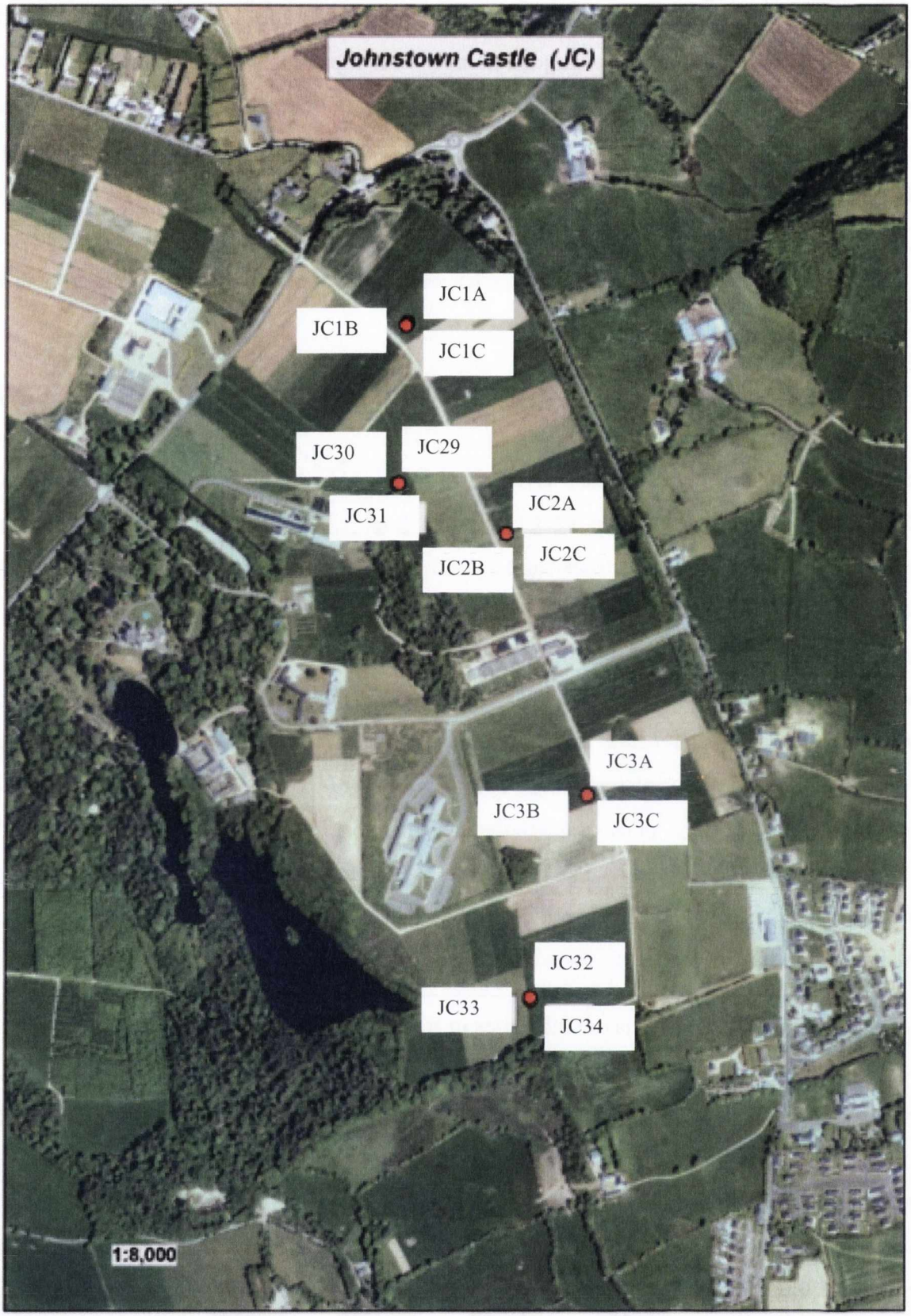


Figure 3.3 Position of the wells on the landscape surrounded by woodland and nearby lakes

Figure 3.4 Position of the wells on the landscape surrounded by grassland

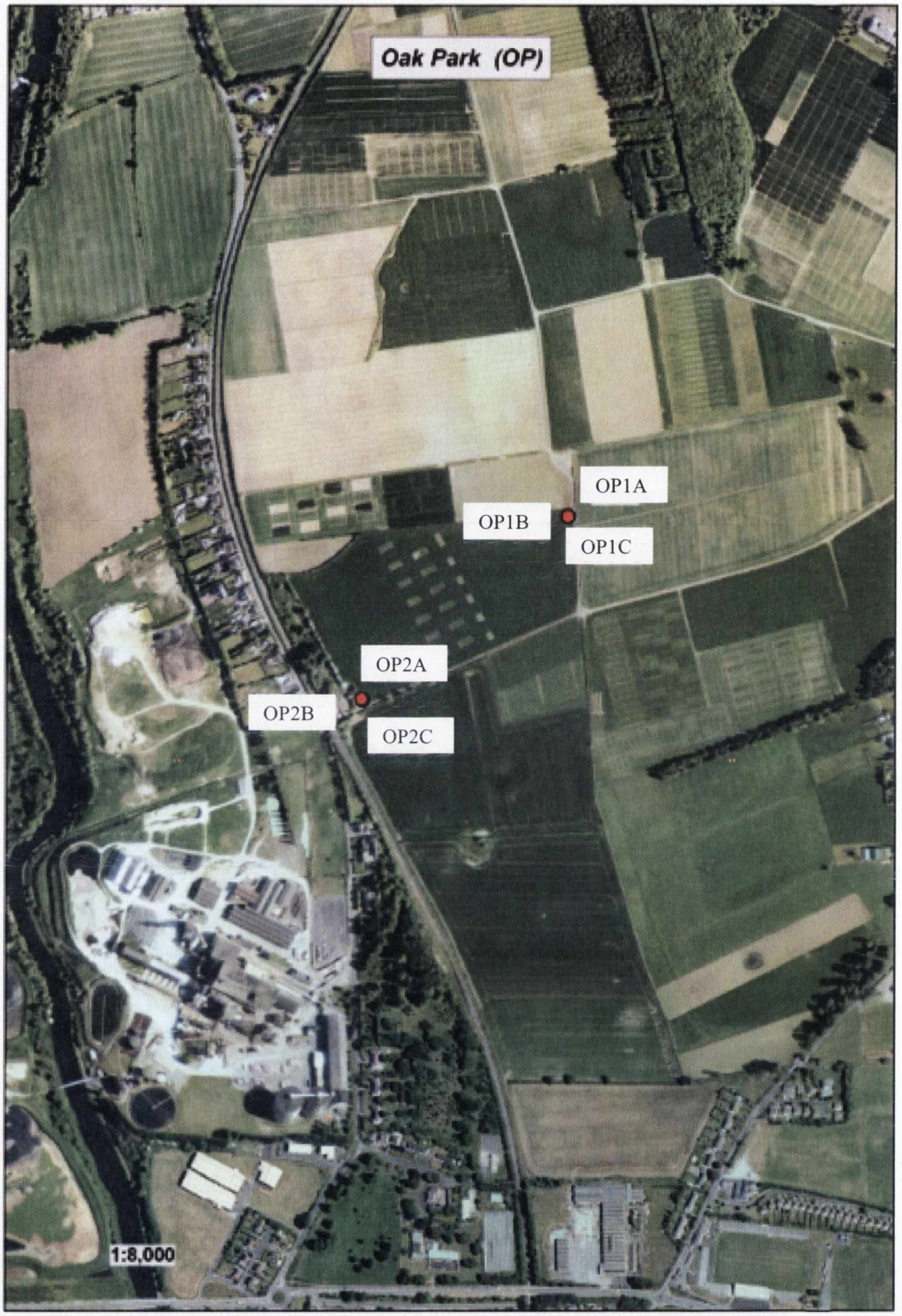


Figure 3.5 Position of the wells on the landscape surrounded by arable land

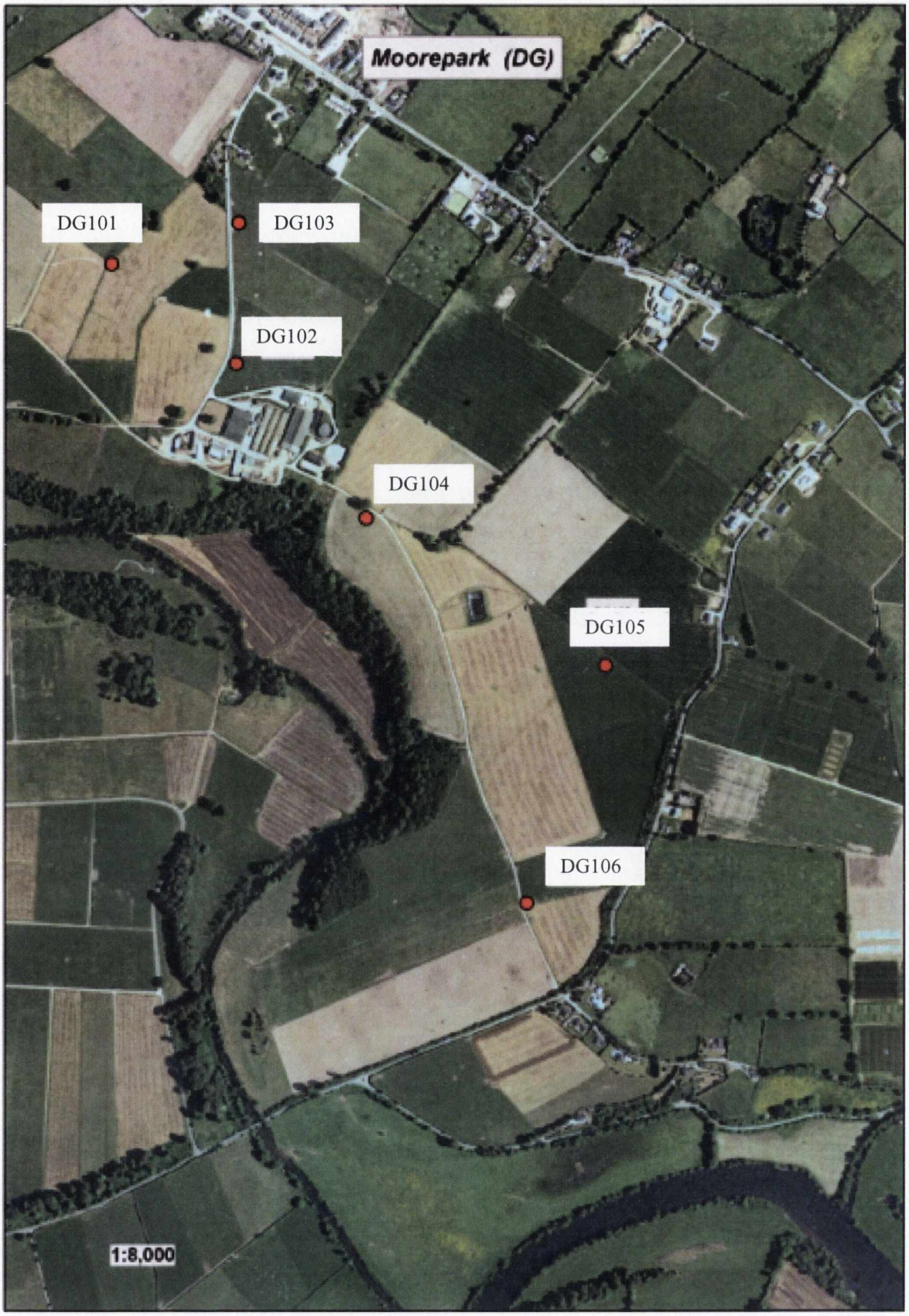


Figure 3.6 Position of the wells on the landscape surrounded by grassland

3.5 Nitrogen input across sites

Nitrogen use efficiency in grassland ranged from 20-30% of applied N and in arable land it is approximately 50%. Even though, the arable site (OP) has very contrasting N application rates (lowest) to the grassland sites. However, the three grassland sites also have different management strategies with regards to grass compositions, N application rates, which resulted in different amount of farm scale N surplus and availability to be leached out to groundwater (Table 3.2). Among the three grassland sites, unlike the JC and DG, SH adopted low input techniques by cultivating leguminous grass (white clover) coupled with other non-legumes. Therefore, TN input at SH is lower than the other two grassland sites (Table 3.2). Moreover, at SH, number of livestock unit (approximately 2.0 LSU per ha) is also lower than JC (approximately 2.3 LSU per ha) and DG (approximately 2.2 LSU per ha). The TN input and surplus in grassland ranged from 213-312 and 137-263 kg N ha^{-1}, respectively which can be compared with the mean N input of 288-335 kg ha^{-1} and surplus of 232 kg N ha^{-1} in Irish grassland for 2003-2006 (Treacy et al., 2008). However, N to be leached rates show quite contrasting values which can be attributed to the variability in N losses via volatilization as NH_3 and denitrification as N_2O and N_2, microbial assimilation and TN build up in soil. For example, soil type at JC and SH is dominated by clay, being with low permeability, enhanced losses of N via denitrification as well as higher TN build up in soil than the DG site. In arable land (OP), as soil is also well drained, tillage enhances nitrification of organic N and thus NO_3^- that exceeded the off take by crops during cropping season and most of the NO_3^- in absence of crops are just ready to be leached out with percolating water. Denitrification losses of N at OP arable land is very low (pers. comm. with Dr. Gary Lanigan, Teagasc) and thus the amount of N leachable is higher in compare to its application rates.

Table 3.2 A simple N balance in four monitoring sites

Site	Land use	N input* (kg N ha^{-1})	N output* (kg N ha^{-1})	N surplus* (kg N ha^{-1})	N to be leached* (kg N ha^{-1})
JC	Grazed grassland	312	69	243	106
SH	Grazed grassland	213	76	137	47
OP	Arable land	150	75	75	70
DG	Grazed grassland	298	35	263	148

*Total N input included fertilizer N, concentrates, atmospheric deposition and biological N_2-N fixation. Total output included milk and meat. Total surplus was calculated by subtracting total output from total input (Scholefield et al., 1991). Total N to be leached was calculated considering N losses via volatilization (NH_3 emission) and denitrification in soil surface (Ryan et al., 2011).

3.6 Soil type and drainage conditions across sites

Major agricultural soil in Ireland is dry and lowland mineral soils accounting for 62% of the agricultural area, while moderately wet mineral soils account for 20% and wet impermeable mineral soils for 17% (Coulter et al., 1996). Soil type and drainage conditions of all selected sites are given in Table 3.3. At JC, it appears that the whole farm is underlain by a fine loamy till which in places is overlain by a stratum of sand of varying thickness. The soil can be grouped as Gleysol or Brown earth and categorized as Humic Gleysol. Overall drainage conditions are moderate to poorly drained, but in a few locations at the down gradient, it has imperfect drainage conditions due to the occurrence of dense clay in subsoils. At SH, drainage is impeded and contributes to waterlogged conditions under high rainfall. The soils on the farm are a mixture of heavy Gleys and Grey Brown Podzolics. The soil has a clay-loam texture, 25% sand and 42% clay in the upper 20 cm with increasingly massive structure and low permeability in lower horizons (poor drainage). At OP, top soils, overlying the glacial till, are sandy loam type with a free draining nature. Below the C horizon, inter-bedded clay lenses reduce the permeability but, overall, permeability is high as the clay lenses are not continuous (Premrov, 2011). Soil type in this area has been reported by Conry (2006) as very gravelly and sandy: >65% of the top soil is sand and >90% of the subsoil is sand and gravel. Soil parent materials are dominantly fluvioglacial sands and gravel, relatively shallow and very vulnerable to leaching (Conry, 2006). At DG, soil texture is sandy loam in top soil and silt loam in subsoils. Soil is relatively free-draining acid brown earth of loam texture. Preferential flow of water in subsoils is likely to take place via fractures in till, which makes water flow even faster than the matrix.

Table 3.3 Soil and subsoil texture type and drainage conditions in soil profile (n = 3) at four monitoring sites JC, SH, OP and DG

Sites	JC		SH		OP		DG	
Depth (m)	Texture type	Drainage	Texture type	Drainage	Texture type	Drainage	Texture type	Drainage
0-0.2	Loam	Moderately drained	Silt loam	Moderately drained	Sandy loam	Well to excessively drained	Sandy loam	Well to excessively drained
0.2-0.6	Silt loam	Moderately drained	Clay loam	Poorly drained	Sandy loam	Well drained	Loam	Well drained
0.6-1.6	Clay loam	Poorly drained	Clay	Poorly drained	Sand clay	Moderately drained	Silt loam	Well drained

3.7 Soil and subsoil physical and chemical properties

A preliminary survey of soil physical and chemical properties was carried out in randomly selected soil profiles, being dug at least three pits (1 m x 1 m x 1.6 m) in each site. Soil bulk (BD) density (Figure 3.7) was higher at OP and DG sites than at JC and SH sites ($p<0.05$), which could be contributed to the dominance of sand particles in soil and subsoils in former sites. The BD increased significantly ($p<0.001$) with depth showing highest in C horizon and lowest in A horizon (Figure 3.7) which was not surprising. Soil pH was highest at OP site (7.5-8.8). At three grassland sites, soil pH was close to neutral such as 5.6-7.0 at JC, 7.0-7.8 at SH and 6.1-7.2 at DG. Considering the implications of soil pH on denitrification, three grassland sites showed a favourable range denitrification to occur (5.5-8.3; Rust et al., 2000) unlike the arable site (pH >8.3).

Soil total carbon (TC) content in up to 1.6 m depth (Figure 3.8) was similar at all sites ($p>0.05$) but it differed significantly among soil depths ($p<0.001$) showing highest in A horizon and lowest in C horizon. It was interesting to note that TC in subsoils (>0.30 m) abruptly increased at OP site, and was higher (in this depth) than all other sites, whereas in top soil it was lower than all other sites. It was possible may be due to the existence of clay band/inter-bedded clay lenses in subsoils. Soil inorganic C content was significantly higher at the OP site than at all other sites (approximately 24% of TC). At the grassland farms, inorganic C content was very negligible (<2% of TC). Total N content was significantly lower ($p<0.001$) at OP site (arable land) than that at all other sites (grassland) (Figure 3.9). The TN content at each site significantly decreased with depth ($p<0.001$) showing the highest in A horizon and lowest in C horizon.

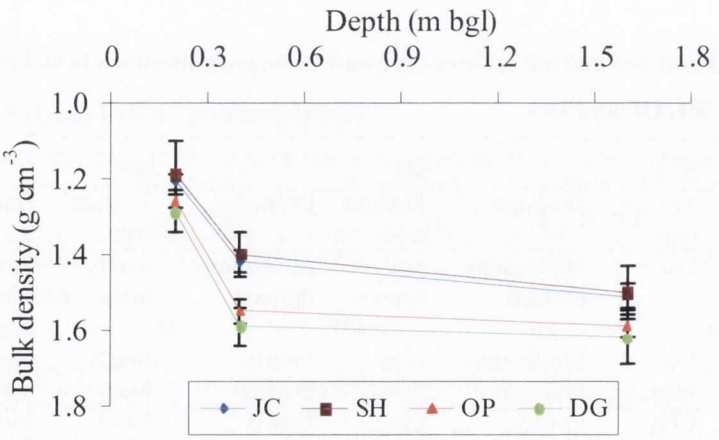


Figure 3.7 Soil bulk density at four different sites and in three depths: 0-.20, 0.40-0.60 and 1.40-1.60 m bgl (below ground level) representing A, B and C horizons

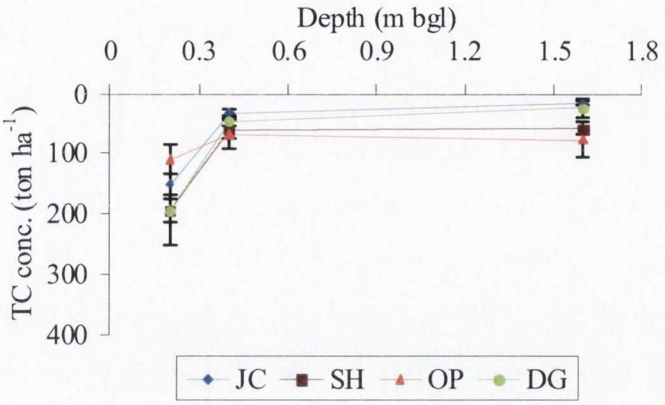


Figure 3.8 Total C (TC) content at four different sites and in three varying depths of soil profile: 0-0.20, 0.40-0.60 and 1.40-1.60 m bgl representing A, B and C horizons

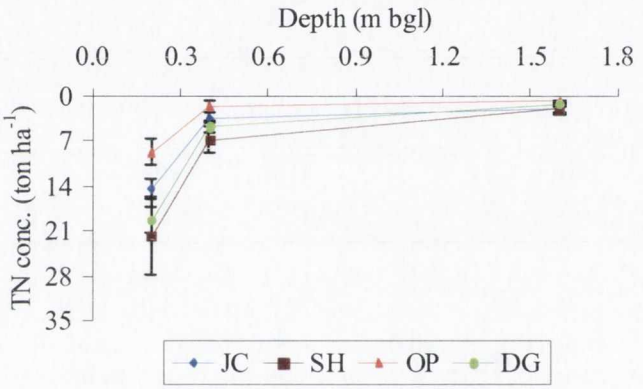


Figure 3.9 Total N (TN) content at four different sites and in three varying depths of soil profile: 0-0.20, 0.40-0.60 and 1.40-1.60 m bgl representing A, B and C horizons

3.8 Hydrogeology of the study sites

The experimental sites were selected with contrasting hydrologic regimes from dryer western part to wetter eastern regions of the country. Mean annual rainfall for preceding 10 years (1999 to 2008) and geology of subsoils and bedrock across sites were given in Table 3.4. At the JC site, the glacial till of dense gravel intermixed with sands and silt with occasional clays is overlying bedrock at approximately 8-10 m bgl of schist, schistose quartzite and Ordovician sediments of sandstones and shales. Mean annual rainfall is highest at this site compared to all other sites. This aquifer is considered to be a poorly productive aquifer. The GWT is comparatively shallow with an average annual fluctuation of 1.5 m bgl. At SH, the aquifer is considered as a poorly productive aquifer. The poorly drained till is overlying Devonian sandstones and mudstones at approximately 6.5 m bgl.

Hydrologically this is comparatively wetter with shallowest GWT (0.2-1.5 m bgl) among the 4 sites. At the OP, shallow sand and gravel aquifer with inter-bedded clay lenses is overlain by sedimentary rocks (gray limestones) - Dinantian Dolomitised Limestones and Dinantian Pure Bedded Limestones at approximately 10-12 m bgl as of drilling log (Appendices 1-4). This type of bedrock covers ca. 17% of the whole country (GSI, 2008). Annual rainfall is low with compare to all other sites. Due to high permeability nature of soils and subsoils, GWT is comparatively deeper (2.5 to 6.0 m bgl). This site is highly vulnerable to groundwater NO_3^- contamination. The DG is the most vulnerable site to groundwater NO_3^- contamination among 4 sites with free draining glacial till of sands and gravel overlying karstified limestones at approximately 10 m bgl. Due the free-draining nature of the aquifer, there is no GWT in the subsoil and glacial till. The GWT is located at 30 m bgl in bedrock zone.

Table 3.4 Subsoil type, bedrock geology and drainage conditions of the study sites

Study site	Glacial till (subsoil)	Bedrock geology	Annual rainfall* (mm)	GWT (m bgl)
JC	Dense gravels intermixed with clay, 0.6 - 10.0 m	Ordovician sediments of sandstones and shales at 10 m bgl; mixed with quartzite.	1053	1.0-2.5
SH	Dense gravels intermixed with dense clay, 0.4 - 8.0 m	Sandstones and some limestones at 8 m bgl.	1004	0.2-1.5
OP	Gravels and sands with interbedded clay band, 0.7 - 14.0 m	Limestones at 12 m bgl; fractures and caverns are present.	890	2.3-6.0
DG	Gravels intermixed with coarse and fine sands, 0.5 - 10.0 m	Grey limestone at 10 m bgl with fractures and caverns.	998	23.0-33.0

* mean for 1999-2008

3.9 Hydrochemistry of the study sites

The hydrochemical properties that are deemed to be important drivers of groundwater denitrification were quite contrasting across sites (Table 3.5). Nitrate concentrations in groundwater were below the recommended threshold level (11.3 mg N L^{-1}) of WHO (1999) and EU WFD (EC, 2000) and nitrate directive at two sites (JC and SH), but it was higher than the threshold value at other two sites (OP and DG). However, it varied from 1 to 26 mg N L^{-1} across sites showing highest at DG and lowest at SH site. The DOC functions as a source of energy for microbial denitrifiers. The DOC concentration was

comparatively low in these aquifers. The DO and Eh indicate the anaerobiocity of groundwater. There are two sites (JC and SH) which seem to be favourable for denitrification to occur because these two sites have low permeability, DO and Eh in groundwater. Therefore, the overall hydrogeochemical conditions of all sites have contrasting conditions to investigate the natural NO_3^- removal capacity and to assess the risk of NO_3^- delivery to the groundwater and surface waters.

Table 3.5 Selected chemical properties of groundwater in study sites at the beginning of the study (Jan, 2009)

Study sites	NO_3^--N (mg L^{-1})	DO (mg L^{-1})	Eh (mV)	DOC (mg L^{-1})
Johnstown Castle (JC)	5-10	<5	120	4
Solohead (SH)	1-3	<5	150	2
Oak Park (OP)	10-15	10-12	200	<1
Dairy Gold (DG)	8-26	10-12	250	<1

3.10 Drilling Log

Top and subsoil types (by finger feel method), colour, moisture content, depth of A, B and C horizons were noted on-site during the boreholing. Approximate GWT depth was identified based on the local hydrological regimes (depth where water struck during boreholing). This information was helpful to locate the screen positions of multilevel wells to be installed so that actual location of GWT and source of water can be identified and availability of water samples can be ensured. Depth of subsoil/glacial till and depth to bedrock were identified and noted on the log and the type of bedrock was also described. Such information can help identify confining layers in aquifer that can be compared afterwards with the measured permeability of wells. Depth was labelled as meter m bgl. Moreover, the sketch of multilevel well construction was drawn with the material used at specific depth (m bgl). The aquifer materials were observed as layered with very heterogeneous mixture of sediments. Groundwater struck alternately showing wet and dry sequence in subsoil and but inside bedrock most of the wells showed a good formation of groundwater. However, these aquifers are generally characterised as poorly productive aquifer. Intact soil/sediments samples in PVC cores (1.0 m long x 0.08 m i. d.) were collected at different depths to determine bulk density, porosity and hydraulic conductivity where feasible. The drilling log maintained on site during boreholing is shown in Appendices 1-4.

CHAPTER 4. DENITRIFICATION POTENTIAL IN SUBSOILS[1]

4.1 An overview of this chapter

Preliminary experiments on subsoil denitrification was conducted in one specific soil and drainage type at one site (Johnstown Castle, JC) to get insights into NO_3^- transformations and N_2O/N_2O+N_2 ratios in subsoils and to elucidate the major factors controlling denitrification. This also has given insights into the abundance of denitrifier genes at varying soil depths. The impact of C on subsoil denitrification in context with its quantity and quality was investigated.

4.2 Introduction

An excess of N in the environment is viewed as an escalating global threat, due to its impacts on groundwater quality and the atmosphere (Stark and Richards, 2008). Soils under grazed grassland often have high concentrations of NO_3^-, arising from the application of mineral fertilizers, slurries, animal excreta and from the native soil organic matter (Foster, 2000). Large amounts of N transferred within the soil system increase the potential and the opportunities for NO_3^- losses (Davies, 2000). The average leaching losses of NO_3^- from terrestrial ecosystems in central Europe is 15 kg N ha^{-1} y^{-1} (Werner, 1994). Nitrate transformation in the root zone is well documented (Ibendahl and Fleming, 2007), but its movement and transformations in prevailing geochemical conditions below the root zone are less well understood (Jarvis and Hatch, 1994). The added NO_3^- can be transported through percolating water and transformed to gaseous forms, thereby leaving agricultural systems, or may be lost through leaching and runoff (Clough et al., 2005). Substantial quantities of dissolved inorganic N, particularly NO_3^-, are exported through low order streams (Alexander et al., 2000). Nitrate contamination of surface water and groundwater is common in watersheds dominated by agricultural activities (Townsend et al., 2003), primarily because of diffuse pollution from intensive farming (Foster and Young, 1980). Denitrification is one of the most important processes that can control the quantity of nitrate available for leaching from soil to water (Jarvis, 2000).

[1] Denitrification potential in subsoils: A mechanism to reduce nitrate leaching to groundwater. Paper published in Agriculture, Ecosystems and Environment, 147, 13-23.

Denitrification is the mainly microbial reduction of NO_3^- to the gaseous products nitric oxide (NO), nitrous oxide (N_2O) or dinitrogen (N_2). This process is an important mechanism for nitrate removal in a variety of suboxic environments (Seitzinger et al., 2006). Some studies have shown that the highest rates of denitrification occur in the upper soil horizon (Clement et al., 2002; Cosandey et al., 2003; Kustermann et al., 2010), the extent of which depends on moisture levels (Khalil and Baggs, 2005). Recently, researchers have found microbial 'hot spots' with significant denitrification activity in patches of organic rich subsoils at depths of several meters (Hill et al., 2004) and in urine treated subsoils (Dixon et al., 2010). Subsoil denitrification has been suggested as an important mechanism for the removal of excess NO_3^- before leaching to groundwater, transport within saturated subsoil zones, or discharge to surface aquifers via subsurface drainage (Fenton et al., 2009a; Sotomayor and Rice, 1996). Denitrification not only serves as a natural pathway for the elimination of excess NO_3^- in soil and water (Ellis et al., 1975), but also contributes to the emissions of N_2O, a potent greenhouse gas (Knowles, 1982) and an indirect contributor to the depletion of ozone (O_3) in the stratosphere (Crutzen, 1970). An interesting feature of denitrification in subsurface soils is that it is likely to be overlooked as a contributor to global atmospheric N_2O concentrations, due to the possible further reduction of N_2O to N_2 under O_2 limited conditions during upward diffusion through the soil profile, if adequate sources of organic C are present (Elmi et al., 2003; Castle et al., 1998).

The beneficial effect to the environment of NO_3^- removal by denitrification depends on the partitioning of its end-products into N_2O and N_2. Knowledge of the denitrification gaseous end-products and the $N_2O/(N_2O+N_2)$ ratio is necessary to assess accurately the environmental consequences of the denitrification process (Elmi et al., 2003), with emphasis on the subsoil environment (Bergsma et al., 2002). The lack of information on N_2 emissions from terrestrial ecosystems not only limits our understanding of its significance as a sink for reactive N, but also impedes the quantification of the process as a whole (Davidsson and Seitzinger, 2006; Groffman et al., 2006) so that N budgets in biogeochemical models are incomplete (Boyer et al., 2006). Depending on the environmental conditions, the mechanisms and magnitude of denitrification losses in subsoils of grazed grassland may, however, deviate considerably from those of other sites warranting further investigation under grassland ecosystems. The relative importance of the denitrification process depends strongly on certain environmental conditions including

O_2 concentration, NO_3^- content and C availability (Tiedje, 1988), though their influences on the mole fractions of N_2O and N_2 in agricultural soils are still under debate, with little consensus (Venterea et al., 2005). Where organic C is added, a significant denitrifying potential may be revealed at depths as great as 7 m (Jarvis and Hatch, 1994; McCarty and Bremner, 1992).

A lack of organic C to provide energy to denitrifiers is usually identified as the major factor limiting denitrification rates (Devito et al., 2000; Pabich et al., 2001). More precisely, the quality and quantity of the C source is most often more important than total organic C due to its variable availability to microbes (Ciarlo et al., 2007). The specific contribution of the different C sources available to denitrifying micro-organisms has not been defined (Beauchamp et al., 1989). Therefore, knowledge of the factors controlling the denitrification process and, more specifically the $N_2O/(N_2O+N_2)$ ratios, are crucial to improve our understanding of the extent of complete reduction of NO_3^- via denitrification occurring in subsoil environments. Concerning health and environmental hazards of NO_3^- and the global warming potential of N_2O, we hypothesized that the addition of a readily available source of C (glucose) would enhance the reduction of N_2O to N_2 in subsoils and show a lower $N_2O/(N_2O+N_2)$ ratio in amended soils than in unamended soils. The main objectives of this section were (a) to measure the potential denitrification rates in subsoils under optimized substrate and moisture conditions and (b) to relate changes in some soil parameters with denitrification rates and with the ratios of denitrification end-products $(N_2O/(N_2O+N_2)$ in sub-surface environments.

4.3 Material and Method

4.3.1 Study site description

Soil samples were collected in January 2008 (winter) from three randomly selected soil profiles in a field under grazed grassland at the dairy farm of Teagasc Environment Research Centre, Johnstown Castle, Wexford, Ireland (52.3342°N, -6.4575°W). The soil textures of a profile up to 1.3 m depth varied from loam to clay loam (Brown Earth) overlying Ordovician sediments of sandstones and shales. Soil physical and chemical properties including the initial nitrate content of three horizons at the experimental site are discussed in chapter 3.5. The average GWT is 1.2 m bgl during winter and 2.0 m bgl during summer. On a yearly average, 50 cows graze this field, which is 48 ha, for a total of

50 days and the total annual N inputs are about 450 kg N ha^{-1} from inorganic fertilizers, animal excrement and N deposition.

4.3.2 Soil sampling

Samples were collected using the standard (Cardenas et al., 2003) methodology for the purposes of this experiment. Intact soil cores (45) were collected from three depths (0-0.10, 0.45-0.55 and 1.20-1.30 m), representing the A, B and C horizons, of the soil profile. Stainless steel cylinders (0.12 m x 0.15 m; Figure 4.1) were manually inserted up to 0.02 m below the top rim of the core (two 0.01 m x 0.01 m steel markers were fixed at two opposite ends of the diameter and at 0.02 m below the top rim of the core) using a percussion hammer into the soil after trimming off the swards to sample the surface/upper horizon (0-0.10 m) and then a hole was dug around the cylinder to assist removal, giving each core a size of 0.1 m x 0.15 m. The two other (deeper) horizons were sampled from the same locations by first removing the soil from the upper horizons. Fine mesh netting was placed over the top and bottom of the cylinders to contain the soil and kept in place using rubber bands at both ends. Soil samples were stored immediately after collection in a cold room at 4°C and transported to Rothamsted Research, North Wyke, UK, in insulated boxes and then stored at 4°C until the commencement of laboratory analysis. Soil corer and incubation vessel are illustrated in Figure 4.1.

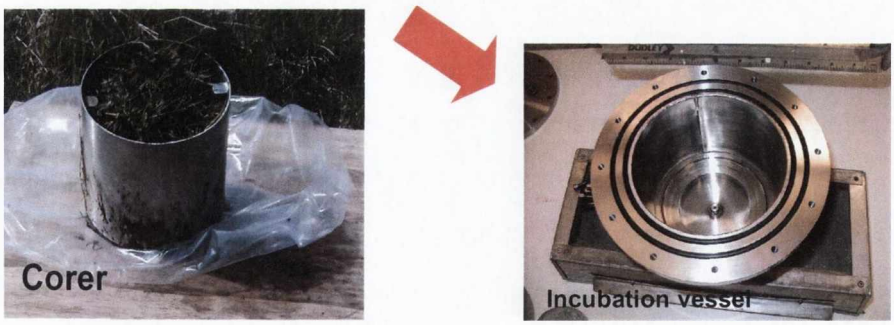


Figure 4.1 Soil corer (left) and incubation vessel (right)

4.3.3 Soil core preparation and amendment

Three sets of 12 cores (3 horizons with 4 replications) were used where all of the soil cores were amended with nitrate (90 mg NO_3^--N kg^{-1} as KNO_3) and the treatments consisted of: (T_1) a control (without C), (T_2) 150 mg glucose-C kg^{-1}, and (T_3) 150 mg DOC-C kg^{-1}. Nitrate was supplied to ensure an adequate source of substrate for denitrification, and the

control was taken to differentiate the effect of the added carbon sources on denitrification. The nitrate rate, assuming negligible inhibition to microbial activity, was selected based on the reports of large leaching losses (50-200 kg NO_3^--N ha^{-1}) from intensively grazed and/or fertilized pasture (Cameron and Haynes, 1986; Jarvis, 2000; Scholefield, 1993; Ledgard et al., 1996). Perego et al. (2011) measured NO_3^--N concentration greater than 100 mg L^{-1} in leachate under maize cropping system. The water soluble organic carbon (WSOC) in the A, B and C horizons was 80, 50 and 24 mg C L^{-1}, respectively. During maximum water holding capacity (MWHC) and FC measurements, water saturation and drain-out may cause losses of indigenous WSOC and NO_3^-. Therefore, an enhanced amount of C and N were added to compensate for losses and also ensured that the denitrifiers were potentially active with readily available substrate.

Each of the three treatment sets of cores was incubated consecutively whilst maintaining exactly the same conditions. During each incubation, 12 soil cores were weighed and placed in a plastic tray of approximately 0.6 m length x 0.5 m width x 0.25 m height and water was added slowly to bring the water level to 3 cm below the top of soil. After 24-h, fine mesh was placed over both ends of the core to prevent soil loss and then the soil core was placed on a fine screen metal sieve fixed to a wooden frame, allowing water to drain out for 30 minutes so as to achieve MWHC of the soil (Karim et al., 1988; Scharenbroach, 2010). After taking weight, the soil cores were kept covered to limit evaporation and were allowed to drain water by gravitation for 48-h and weighed again to estimate the field capacity (FC) of the soil (Karim et al., 1988; Scharenbroach, 2010). The amendment solutions were prepared with an amount of water required to maintain the soil WFPS levels at a moisture content of 3% above the moisture content at FC: ca. 80, 85 and 88% for the soils collected from A, B and C horizons, respectively. Potential denitrification rates require approximately anaerobic conditions (greater than 80% WFPS). Because the existing field conditions would have higher O_2 concentrations in the top soil than the subsoils, the WFPSs imposed for this study were chosen to reflect these relative differences as considered most closely appropriate.

4.3.4 Preparation of dissolved organic C (DOC) solution used

Surface soils (1 kg) from grazed grassland were collected; herbage, roots, stones and other extraneous materials were removed. Subsequently, 100 g soil was placed into a 500 ml plastic bottle and 150 ml deionised water was added (1:1.5 v/v ratio). The bottle was

shaken mechanically for 1-h. The supernatant was removed following sedimentation, and was centrifuged for 30 minutes at 2500 rpm; filtered using filter paper (Whatman No. 41) and DOC was measured using a TOC analyser (TOC-Vcph/cpn; Shimadzu Corporation, Kyoto, Japan). The NO_3^- and NH_4^+ concentrations being 1.5 and 2.2 mg kg^{-1}, respectively were negligible, but were deducted from the amendment added to soil cores.

4.3.5 Soil core pre-incubation, incubation and data recording

The denitrification study was carried out by incubating the soil cores at 15°C, for 17-day, in an automated laboratory incubation system installed at Rothamsted Research, North Wyke (Cardenas et al., 2003; Scholefield et al., 1997). The incubation system (Figure 4.2) comprised a 1.3 m^3 temperature controlled cabinet containing 12 incubation vessels (each fitted with an amendment vessel) and gas lines. Headspace temperatures inside the vessels were logged hourly. Each of 12 soil cores was then placed inside a cylindrical incubation vessel to an exact fit. A mixture of *He* + O_2 was passed through the soil core (via the base of the vessel) in order to purge (flow-through mode) the soil atmosphere, headspace and all N_2 gas lines for 24-h. Flow rates of a *He*+O_2 mixture (20 ml min^{-1}) were regulated using mass flow controllers to provide an O_2 concentration of ca. 20% (Cardenas et al., 2003; Scholefield et al., 1997). The *He*+O_2 mixture was then directed to the vessel via the lid (flow-over mode) after reducing the flow rate to 10 ml min^{-1} and O_2 level to 15% for 72-h. The effluent gases from each vessel were passed through an outlet in the lid of the incubation vessels to an actuated 16-port selection valve to split and direct the gas stream from each outlet column to a gas chromatography (GC) (automatic sample feeding). Flow-over continued for 72-h until the measured N_2 levels had reached a baseline. After replacement of the atmosphere within the soil cores, amendments were added via a secondary vessel, fitted to the centre of each lid, by flushing with *He* (to avoid any atmospheric N_2 contamination). The amendment in the soil cores was found to be well distributed based on subsequent analyses of nine sub-samples from each core from three vertical and three horizontal sections. The technique allowed the direct and independent measurement of N_2O and N_2 fluxes from each incubation vessel, which permitted an exact measurement of denitrified gas concentrations. Continuous recording of N_2O and N_2 concentrations were automated at a frequency of approximately 12 measurements per day using Shimadzu GC throughout the experiment. N_2O was detected by Electron Capture Detector (ECD) with separation achieved by a stainless steel packed column (2 m long, 4 mm bore) filled with 'Porapak Q' (80-100 mesh) and using N_2 as a career gas. N_2 was

detected by *He* Ionization Detector (HID) with separation achieved by a PLOT column (30 m long, 0.53 mm i. d.), with He as the carrier gas. The software 'Kontron' (Kontron Electronic, Munich, Germany) was used to measure the concentration of effluent gases.

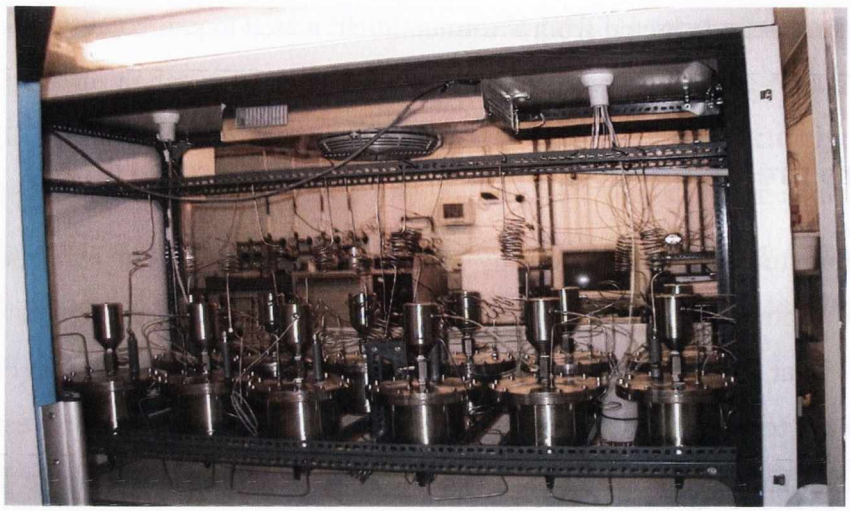


Figure 4.2 Automated incubation chamber with incubation and amendment vessels

Scholefield et al. (1997) found that this technique is particularly suited to an investigation into the effects of O_2 concentration *per se*. They observed O_2 concentrations negatively correlated with WFPS in the automated technique of a denitrification study. Therefore, higher WFPS in subsoil horizons (85-88%) than in A horizon (80%) indicated lower O_2 contents. The technique prevented further O_2 diffusion from headspace into soil cores, because no changes in the estimated water contents (measured at the initial, highest peak and end of the experiment) was observed. This provided evidence that no air exchange into the soil cores occurred during the incubation period and predicted that microbiological compositions remained relatively unaltered throughout the experimental period.

4.3.6 Physical and chemical analyses

In addition to the three treatment sets of cores (36 in total), an additional three cores from each horizon (9 cores) were sampled before pre-incubation. Another three cores were removed from incubation on the day following the highest recorded N_2O peak and before the N_2 peak was attained (this left three replicates out of the four original treatment sets to continue until the end of the incubation). At the end of each experiment, all soil cores were prepared for physical and chemical analyses. Pre-incubation, at peak N_2O and N_2 emission points and at the end of incubations, soil sub-samples were taken for microbial analysis, as described by Barrett et al. (2010). Soil moisture content was measured gravimetrically after

drying for 24-h at 105°C. Dry bulk density (BD) was determined by a soil core method, using the oven dry weight of soil and the known volume of the soil corer. Soil mineral N as NH_4^+ and NO_3^- were analysed using an Aquakem 600 Discrete Analyser (Askew and Smith, 2005b; Standing Committee of Analysts, 1981) after extraction with 2 M KCl in 1:2.5 (w:w) of soil and KCl solution. The WSOC was analysed on a TOC Analyser (TOC-Vcph/cpn; Shimadzu Corporation, Kyoto, Japan) after extraction with deionised water (soil water ratio 1:2.5). The WSOC extracts were first used to measure pH and then centrifuged at 1500 rpm for 30 min and then filtered on a 0.45 μm filter. Soil total organic C and N were determined by dry combustion analysis (Leco CNS 2000 analyzer; Leco Corporation, USA).

4.3.7 Calculation of potential denitrification

Denitrification potential is defined as the denitrification rate under anaerobic conditions with abundant NO_3^- (Aulakh et al., 1992) and available organic C as an energy source for denitrifying organisms (Well and Myrold, 2002). N_2O and N_2 fluxes (mg N kg^{-1} dry soil d^{-1}) were calculated from the concentrations continuously measured by the GC during the entire incubation period. Approximately 12 measurements were recorded per sample per day and averaged to express flux as mg kg^{-1} d^{-1}. Denitrification rates and total denitrification (TDN) losses of added N were calculated by summing N_2O+N_2. The N_2O mole fractions were calculated using N_2O fluxes and the total fluxes of N_2O and N_2 $[N_2O/(N_2O+N_2)]$. All the calculated results were then compared for three soil depths and treatments.

4.3.8 Statistical methods

All statistical analyses were performed using SPSS 16 (SPSS Inc. USA). Because variables resulted log normally distributed, a log transformation was applied. The residual checks confirmed the assumptions of the analyses and the homogeneity of variances within each treatment. A 2-way ANOVA was carried out to distinguish treatment and depths effects on the data at maximum fluxes, mean and cumulative emissions of N_2O, N_2, N_2O+N_2 and on the $N_2O/(N_2O+N_2)$ ratios over the incubation period with treatment and soil depths as fixed factors following univariate analysis under a General Linear Model. Multiple comparisons test between individual treatment and depth effects were carried out using the Bonferroni Post Hoc test. Simple and multiple linear regressions (stepwise) analyses using the data

points at the highest flux stage were carried out to test relationships between potential denitrification rates and soil properties (soil pH, NH_4^+, NO_3^-, total N, organic N, inorganic N, WSOC, total C and organic C) after converting all non-normal data to log-transformed data. For correlation and regression study we used soil cores at maximum emissions under all treatments because our interest was to see the changes in some soil physico-chemical properties at the very moment of maximum denitrification. For this, we removed additional soil cores (one or two for each treatment and depth) during maximum denitrification from the incubation chamber for each depth in each run. A statistical probability of $p<0.05$ was considered significant for all tests.

4.3.9 Results of N_2O and N_2 fluxes

Mean fluxes of N_2O varied significantly between treatments ($p<0.01$), soil horizons ($p<0.001$) and the interaction of treatments and soil horizons ($p<0.05$) (Figure 4.3). The maximum N_2O fluxes that appeared during the incubation period varied significantly between treatments ($p<0.001$) and depths ($p<0.001$). The nitrate + glucose-C (T_2) and nitrate + DOC (T_3) treatments showed the highest peaks for N_2O fluxes on day 1 after the amendment in the A horizon (9.91 and 7.22 mg N kg^{-1} dry soil for T_2 and T_3, respectively). Though smaller (1.28 mg N kg^{-1} dry soil), the maximum emissions in the nitrate only (T_1) treatment was delayed for two days. The maximum peaks were several-fold lower in the subsoils (B and C horizons), ranging from 0.07-0.22, 0.20-0.44 and 0.47-1.04 (mg N kg^{-1} dry soil) for T_1, T_2 and T_3 treatments respectively, compared with the A horizon and observed between day 4 and 8 of incubation. Similarly, mean N_2O fluxes over the incubation period were significantly ($p<0.001$) greater in the A horizon (0.77 to 2.38 mg N kg^{-1} d^{-1}) than in the subsoil horizons (0.07 to 0.54 mg N kg^{-1} d^{-1}); the lowest being in the C horizon. Overall, the soil cores amended with NO_3^- only (T_1) displayed significantly ($p<0.01$) lower cumulative N_2O emissions than the T_2 and T_3 treatments, whereas it was consistently ($p>0.05$) higher in the treatment with glucose-C (40.5 mg N kg^{-1}) than with DOC (23.8 mg N kg^{-1}). Despite low emissions, subsoils that received DOC enhanced N_2O emissions but did not differ significantly with those that received glucose-C (Table 4.1).

Table 4.1Mean and cumulative N_2O and N_2 fluxes/emissions at various soil horizons as affected by N and C sources during the 17-day incubation period (n=3).

Treatment	Soil Horizon	N_2O		N_2	
		Cumulative emissions (mg N kg^{-1})	Flux rate (mg N kg^{-1} d^{-1})	Cumulative emissions (mg N kg^{-1})	Flux rate (mg N kg^{-1} d^{-1})
T_1: NO_3^- only	A	13.05	0.77	9.35	0.55
	B	1.27	0.07	9.01	0.53
	C	0.67	0.04	2.15	0.13
T_2: NO_3^-+ Glucose-C	A	40.52	2.38	13.56	0.80
	B	2.54	0.15	23.60	1.39
	C	0.99	0.06	15.70	0.92
T_3: NO_3^- + DOC	A	23.81	1.40	16.69	0.98
	B	9.21	0.54	16.30	0.96
	C	1.59	0.09	13.90	0.82

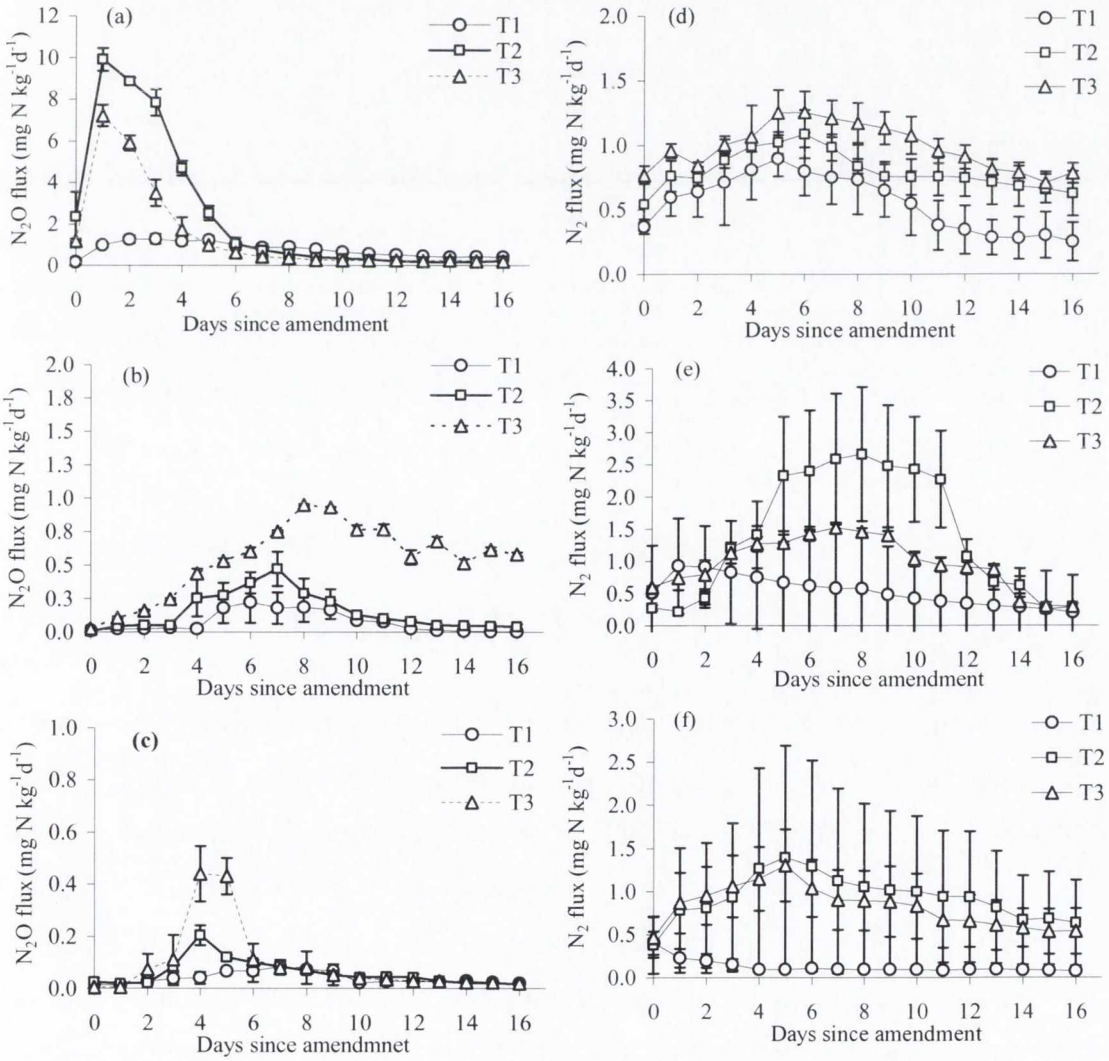


Figure 4.3N_2O and N_2 fluxes from three different soil horizons, A (a, d); B (b, e) and C (c, f) as influenced by nitrate only (T_1); nitrate+glucose C, (T_2) and nitrate+DOC, (T_3).

The treatment and soil depth had pronounced effects on the time course of N_2 fluxes (Figure 6.1). In the A horizon, the highest peak was observed on day 6 after amendment with nitrate + glucose-C and nitrate + DOC (1.03 and 1.29 mg N kg^{-1} dry soil in T_2 and T_3, respectively) and on day 5 of incubation when treated with nitrate only (0.96 mg N kg^{-1} dry soil). In subsurface horizons, the highest peaks were observed on day 1 after amendment with nitrate only (0.66, and 0.38 mg N kg^{-1} dry soil at B and C horizons), but it was delayed by 4-7 days in the treatment that had C. The mean N_2 fluxes only differed significantly ($p<0.05$) between the A and C horizons. In the A horizon, it ranged from 0.55 mg N kg^{-1} d^{-1} in T_1 to 0.98 mg N kg^{-1} d^{-1} in T_3 (Table 6.2). In the C horizon, it varied from 0.13 mg N kg^{-1} d^{-1} in T_1 to 0.92 mg N kg^{-1} d^{-1} in T_2. Added C did not affect the mean N_2 flux significantly ($p>0.05$). The T_2 treatment showed consistently higher emissions than T_3 though the difference was not significant. In contrast to the subsoil horizons, cumulative N_2 emissions in the A horizon were higher with added DOC than with added glucose-C (Table 4.1).

4.3.10 Total denitrification rates and the losses of added nitrogen

The TDN (N_2O+N_2) rate significantly ($p<0.05$) differed with regards to soil depth and treatments (Figure 4.4a). Cumulative TDN emissions were significantly higher in the A horizon than in the B ($p<0.05$) and C ($p<0.01$) horizons, but the latter two were not statistically different from each other. Considering multiple comparisons between the treatments, the soil cores amended with nitrate alone (T_1) showed significantly ($p<0.01$ for T_2 and $p<0.05$ for T_3) lower TDN emissions (ca. 22.4, 10.3 and 2.82 mg N kg^{-1} from A, B and C horizons, respectively) than the same horizons amended with either glucose-C (ca. 54.1, 26.2 and 16.7 mg N kg^{-1} for A, B and C horizons, respectively) or DOC (ca. 40.5, 25.5 and 15.5 mg N kg^{-1} from A, B and C horizons, respectively). The treatment and soil depth significantly affected the percentage losses of added N (Figure 4.4b). The loss of added NO_3^--N from T_1, T_2 and T_3 treatments, respectively were significantly greater in the A horizon (ca. 25, 60 and 45%) compared with B ($p<0.05$) (ca. 12, 29 and 29%) and C ($p<0.01$) (ca. 3, 20 and 18%) horizons and the B and C horizons also differed significantly ($p<0.05$). Addition of C significantly increased N losses in T_2, nitrate + glucose-C ($p<0.05$) and T_3, nitrate + DOC ($p<0.01$) compared with the T_1, nitrate only treatment. There were no significant differences between the two C sources.

4.3.11 Nitrous oxide mole fractions at various soil depths

The mole fractions of N_2O varied significantly ($p<0.05$) with soil depth but did not differ significantly to either added N with or without C sources (Figure 4.4c). The A horizon had significantly ($p<0.05$ for B and $p<0.01$ for C horizons) greater N_2O mole fractions (0.58-0.75) than the subsoil horizons (0.06-0.36).

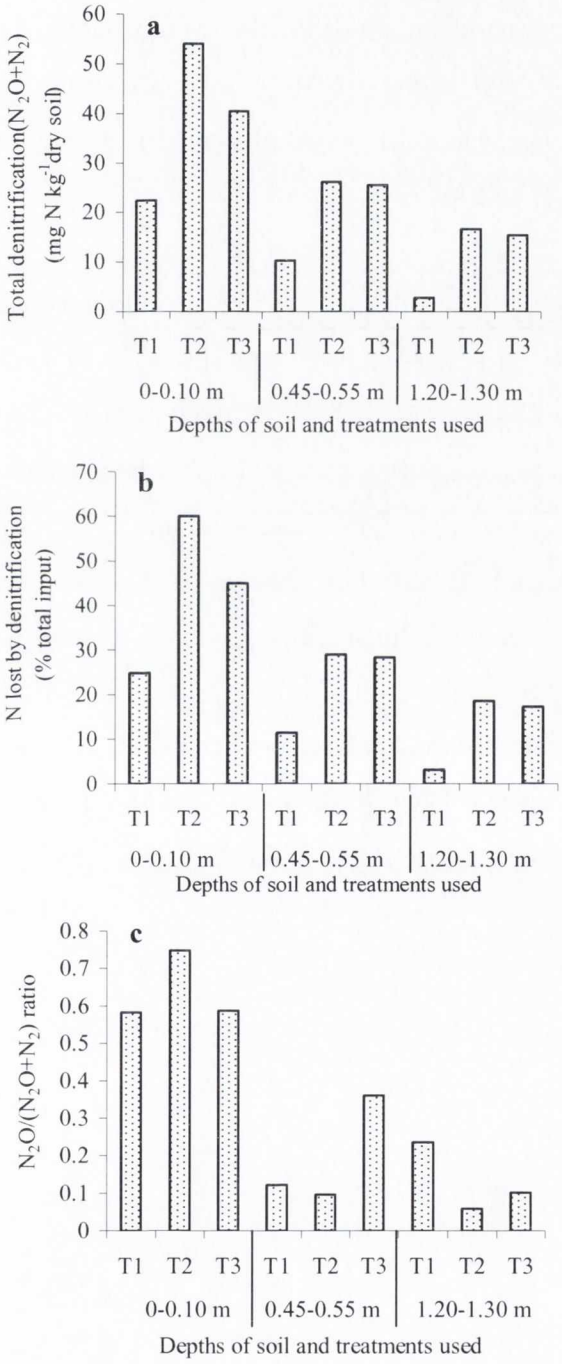


Figure 4.4Cumulative denitrification (N_2O+N_2) (a), percentage losses of the applied N (b) and N_2O mole fractions (c) from three different treatments (see text) and soil horizons during the 17-day incubation period.

4.3.12 Relationship between denitrification and soil properties

Pearson correlation coefficients between denitrification end-products and all the soil-related controlling factors with their levels of significance are shown in Table 6.3. As expected, there was a significant ($p<0.001$) positive correlation between N_2O flux and TDN rates with $R^2 = 0.95$. The N_2O mole fractions were also positively and significantly correlated with TDN rates and N_2O flux, giving R^2 values of 0.50 and 0.55, respectively. The estimated coefficients of soil physico-chemical properties were selected as significant explanatory variables for the models that had the best fit to predict the observed flux following stepwise multiple linear regressions of potential denitrification rates and N_2O mole fractions during the incubation (Table 4.2).

Considering the three soil horizons, a significant positive correlation was observed between N_2O flux and total organic carbon ($p<0.001$) and soil total N ($p<0.05$) but a significant negative correlation was observed with NO_3^--N ($p<0.001$) at the moment of maximum emission. The N_2 flux was significantly positively correlated with TON ($p<0.01$) and negatively with NO_3^--N ($p<0.05$). The regression model developed for the estimation of N_2 emissions explained only 45% of the variances of N_2 flux (Table 4.3). The TDN (N_2O+N_2) showed a significant positive linear relationship with total C ($p<0.001$), but a significant negative relationship with NO_3^--N ($p<0.01$). The empirical model developed through stepwise multiple regression analysis for TDN rates also included these variables and explained 76% (adjusted $R^2=0.76$) of variances (Table 4.3). Strong positive relationship was observed for N_2O mole fraction ($N_2O/(N_2O+N_2)$) individually with total C ($p<0.01$) and pH ($p<0.01$).

Table 4.2 Pearson's correlation coefficients 'r' between N_2O, N_2, N_2O+N_2 and $N_2O/(N_2O+N_2)$ ratio and measured soil properties; soil properties were expressed as ‡mg kg^{-1} dry soil except pH; denitrification rates were expressed as ‡mg kg^{-1} dry soil d^{-1} except the N_2O/TDN

	pH	WSOC ‡	TOC ‡	TC	NH_4^+-N‡	NO_3^-N ‡	TIN‡	TORG-N‡	TN	N_2O ‡	N_2‡	TDN ‡	N_2O/ TDN‡
pH	1												
WSOC‡	0.38ns	1											
TOC‡	0.92**	0.47*	1										
TC	0.89**	0.44*	0.94**	1									
NH_4^+-N‡	0.44*	0.34ns	0.54*	0.62**	1								
NO_3^--N‡	0.19ns	-0.06ns	-0.07ns	-0.02ns	-0.12ns	1							
TIN‡	0.45*	0.24ns	0.47*	0.39ns	0.26ns	0.16ns	1						
TORG-N‡	0.90**	0.43*	0.94**	0.99**	0.61**	-0.02ns	0.36ns						
TN	0.90**	0.43*	0.95**	0.99**	0.62**	-0.01ns	0.38ns	0.99**	1				
N_2O‡	0.47*	0.43*	0.64**	0.75**	0.56**	-0.59**	0.14ns	0.74**	0.74**				
N_2‡	0.43*	0.22ns	0.48*	0.51*	0.35ns	-0.43*	-0.08ns	0.52*	0.52*	0.53*	1		
TDN‡	0.57**	0.38ns	0.66**	0.75**	0.57**	-0.56*	0.03ns	0.75**	0.74**	0.85**	0.86**	1	
N_2O/TDN‡	0.58*	0.36ns	0.47*	0.58**	0.42ns	-0.40ns	0.20ns	0.56**	0.56**	0.90**	0.13ns	0.52*	1

WSOC, TOC, TC, TIN, TORG-N, TN, and TDN stand for water soluble organic C, total organic C, total C, total inorganic N, total organic N, total N, and total denitrification (N_2O+N_2), respectively; **p <0.01; *p<0.05; and ns, non significant; ‡ln=natural logarithm

Table 4.3 Estimated coefficients of physico-chemical properties selected as significant explanatory variables using a stepwise procedure for models of denitrification products and ratios (n=27)

Denitrification products and ratio‡	Equation element‡	Estimate	s. e.	Significance	Partial R^2
	(mg kg^{-1})				
lnN_2O	Intercept	11.769	2.208	***	
	lnTOC	0.002	0.001	***	0.57
	$lnNO_3^-$-N	-1.776	0.292	**	0.22
	TN	0.715	0.207	*	0.09
lnN_2	Intercept	2.036	1.040	**	
	TORG-N	0.001	0.001	**	0.27
	$lnNO_3^-$-N	-0.581	0.239	*	0.18
lnTDN	Intercept	3.040	0.892	***	
	TC	0.002	0.001	***	0.56
	$lnNO_3^-$-N	-0.800	0.205	**	0.22
$Ln(N_2O/(N_2O+N_2))$	Intercept	3.200	1.135	**	
	TC	0.001	0.001	**	0.34
	pH	0.900	0.232	**	0.29

‡ln = unit in natural logarithm; TN, TOC, TC and TORG-N represent respectively, total N, total organic C, total C and total organic N

4.4 Interpretation of the results

4.4.1 N_2O and N_2 fluxes

The maximum peaks for N_2O fluxes in the A horizon appeared on 1 day after the amendment was applied, in all treatments except the cores that received nitrate alone. In the other two subsoil horizons (B and C), the maximum peaks appeared on day 4 and 8 after amendment, regardless of the treatments applied. The A horizon time course for the peaks was slightly different from those observed by Scholefield et al. (1997), who reported the highest peak for N_2O in surface soil on day 2, i.e. one day later than we observed. This might be due to the different nutrient rates (nitrate 50-100 kg ha^{-1}, glucose 394 kg ha^{-1}) and soil conditions they used e.g. pH 5.1. However, in the A horizon cores, the highest peaks of N_2 appeared 3- 4 days later than (on day 5 and 6 after the amendment) the highest peaks of N_2O regardless of the treatments. The time course for A horizon N_2 peaks were quite similar to the finding of Scholefield et al. (1997) for the appearance of the N_2 peaks. In the

A horizon, the N_2O and N_2 emissions for the consecutive days of their peaks were also in agreement with the findings of Cardenas et al. (2003) and Miller et al. (2009), where the highest N_2O and N_2 peaks appeared on day 1 and 3 after amendment, respectively. In the two subsoil horizons (B and C) the N_2 peaks appeared only one day later than the N_2O peaks and the addition of C sources delayed the appearance of peaks for two to three days.

N_2O emissions were observed at lower concentrations in the C horizon, compared with the shallower A and B horizons. Li et al. (2002) also reported N_2O production in the B and C horizons (0.016-0.233 $\mu g\ L^{-1}$). The decrease of denitrification rates with increasing soil depth has also been observed in previous studies (e. g. Dambreville et al., 2006; Dixon et al., 2010). The underlying causes of higher N_2O fluxes in the A horizon is probably due to the higher total organic C sources and greater denitrifier abundances compared with subsoil horizons. The N_2O emissions from the treatment, without the addition of C, were very similar to those reported by Castle et al. (1998), of 0.103-0.672 mg N kg^{-1} d^{-1}, and by Richards and Webster (1999) of 0.029-0.185 mg N kg^{-1} d^{-1} in subsoils (0.6 to 1.4 m depths). The addition of C as either glucose and DOC increased N_2O emissions by 45 and 67% in the A horizon; by 50 and 150% in the B horizon and by 25 and 55% in the C horizon, respectively. Our results also agree with other laboratory experiments, which reported between 30 and 50% of applied N lost as N_2O (Cardenas et al., 2003; Miller et al., 2009; Pfenning and McMahon, 1996) stimulated by C addition. In the A horizon, added glucose-C increased N_2O emissions more than added DOC, although not in the subsoil horizons. McCarty and Bremner (1992) found that DOC is rapidly metabolized by the microbial community. Contrasting effects of the added C sources on N_2O emissions in the top soil and subsoils might be attributed to the differences in the native organic C pools, water-holding capacity, pH, bulk density, and mainly fungal and bacterial community structure dynamics (Anderson and Peterson, 2009; Laughlin and Stevenson, 2002).

Higher N_2 flux from the C horizon than the A horizon could possibly be due to the higher bulk density and WFPS in C horizon. A higher bulk density will alter pore geometry and connectivity resulting in higher N_2O generation and a longer residence which may allow a more complete reduction of N_2O to N_2 (Jacinthe and Dick, 1997; Elmi et al., 2003). The absence of treatment effects with the application of a high levels of NO_3^--N may be explained by the finding that high NO_3^- concentrations can inhibit the reduction of N_2O to

N_2 (Blackmer and Bremner, 1978), which might mask the influence of added N and C on N_2 fluxes. By contrast, Miller et al., (2009) observed that C availability in soil could promote the reduction of N_2O to N_2. Scholefield et al. (1997) postulated that with increasing concentration of soil NO_3^-, denitrification changes from being dependent on NO_3^-, with first order kinetics, to being independent of NO_3^- that is, following zero order kinetics. Interestingly, glucose-C showed consistently more potential to enhance further reduction of N_2O to N_2 in the top soil, as it provided lower N_2O but higher N_2 than measured following DOC application; a situation which was reversed in the subsoils. This may be due to the variability in effects of glucose-C and DOC on microbial functions, as fungi were reported to retard further reduction of N_2O to N_2 (Laughlin and Stevens, 2002).

4.4.2 Total denitrification (TDN) rates

The TDN rates decreased with increasing soil depth indicating that topsoil bio-, physico-chemical conditions were more favourable than subsoils for potential denitrification to occur. This suggestion was supported by analysis of the diversity and abundance of microbes (*Bacteria* and *Archaea*) harbouring denitrifying functional genes (*nirK* - nitrite reductase that contains copper; *nirS* - nitrite reductase that contains heme c and heme d_1; *nirK + nirS combinedly termed as nir (nitrite reductase); nosZ* - nitrous oxide reductase), within each of the three soil horizons and the three separate sampling stages e.g. before incubation, following the highest peak of N_2O and at the end of incubation, which was carried out by Barrett et al. (2010). Briefly, the authors reported a significantly higher abundance of denitrifying functional genes and bacteria in the A horizon compared with the B ($p<0.01$) and C ($p<0.01$) horizons, and a higher *nosZ* gene abundance in the subsoil horizons than in the A horizon ($p<0.001$), irrespective of the treatments applied. Between the two subsoil horizons, the C horizon had significantly lower denitrifying functional and bacterial genes than the B horizon ($p<0.01$). The concentration of archaeal gene copy numbers was similar across all horizons. In the A horizon, the analyzed gene copy numbers were 10^5-10^6 genes g-^{1} soil for *nirK*, 10^5-10^7 genes g^{-1} soil for *nirS* and 10^4-10^5 for *nosZ*. In the subsoil horizons the analysed copy number were 10^4-10^6 genes g^{-1} soil for *nirK*, 10^4-10^7 genes g^{-1} soil for *nirS* and 10^5-10^6 genes g^{-1} soil for *nosZ* (Barrett *et al.*, 2010). Frey et al. (1999) also reported a significantly higher total microbial biomass (bacterial and fungal)

in the top soil layer than in the lower layer. The treatment, which received NO_3^- only, registered lower losses of the applied N than the treatments receiving NO_3^- coupled with either glucose-C or DOC, with consistently lower losses found with DOC addition. Analysis of soil parameters at the end of incubation showed a recovery of 20% of the added nitrate in soil cores (e.g. in the A horizon with T_2 where 61% nitrate was denitrified), which might have been denitrified if the incubation time was extended, but the remaining 19% of added nitrate might be immobilized due to C addition. The NH_4^+ concentrations at the end of incubation in all soil cores were approximately similar to the initial concentrations, indicating no evidence of dissimilatory nitrate reduction to ammonium. Stimulus of subsoil denitrification by added C was reported from laboratory (Khalil and Richards, 2010) and field studies (Weier et al., 1993). Our results of TDN (15.49-26.15 mg N kg^{-1} dry soil equivalent to the TDN rates of 0.91-1.54 mg N kg^{-1} d^{-1}) in the subsoil horizons (clay loam) under adequate C sources were similar to other studies. Jarvis and Hatch (1994) reported potential denitrification rates of 1.0 mg N kg^{-1} dry soil d^{-1} in grassland subsoils (loam) while Yeomans et al. (1992) found 1.4-5.1mg N kg^{-1} dry soil d^{-1} in subsoil with a non-limiting C source. Khalil and Richards (2010) reported a small denitrification capacity in subsoils (C horizon; sandy clay loam to clay loam) of grazed pasture (0.03-0.05 mg N kg^{-1} soil d^{-1}) and its potential was found to be significantly higher in subsoils of grazed ryegrass than clover-grass (1.15 vs. 0.50 mg N kg^{-1} soil d^{-1}).

4.4.3 N_2O mole fractions ($N_2O/(N_2O+N_2)$) at various soil depths

In the A horizon, N_2O was the dominant denitrification end product (58-75%) that increased by 2 to 30% with the addition of C sources. The N_2O mole fractions were significantly lower (6-36%) in the two deeper soil horizons, compared with the A horizon, suggesting more complete reduction of N_2O to N_2. As the N_2O mole fraction did not differ significantly between the treatments, but differed significantly between the soil horizons, it can be postulated that N_2O mole fraction was a function of soil depths which had different WFPS and thus different O_2 concentrations. The N_2O-to-N_2 ratios do generally decrease with increasing WFPS and from an experiment in grassland soil, Scholefield et al. (1997) reported that with increasing WFPS from approximately 70 to 90%, there was a greater than 50-fold increase in denitrification. It is well known that denitrification is inhibited

progressively by increasing O_2 concentrations in the soil, probably linked to the acute sensitiveness to the nitrate reductase enzyme system, and that N_2O-to-N_2 ratios decrease with increasing soil water content (Knowles, 1982). Even trace amounts of O_2 can inhibit nitrous oxide reductase activity (Zumft, 1997; Knowles, 1982). Therefore, the decrease in $N_2O/(N_2O+N_2)$ with increasing soil depths may be attributed to the reduction of N_2O to N_2 at increased moisture levels. Similarly, Ciarlo et al. (2007) found highest N_2O emission in 80% WFPS compared with 40, 100 (saturated) and 120% (over-saturated with about 2 cm overlying surface water layer) and $N_2O/(N_2O+N_2)$ was lowest at 120% WFPS. This finding is in agreement with Granli and Bockman (1994) who reported that within the range 60-90% WFPS, aeration could increase the proportion of N_2O produced by denitrification.

Higher bulk density with correspondingly lower permeability in subsoils than in the A horizon can increase the residence time of N_2O by slowing down diffusion rates and eventually reduces N_2O proportion. Further, the denitrified N_2O gas formed in the subsoil could have a large potential to undergo further microbial reduction to N_2 during the slow diffusion process across the soil profile (Castle et al., 1998; Ciarlo et al., 2007). Farquharson and Baldock (2008) suggest that the amount of N_2O that moves through the entire denitrification pathway to N_2 depends on the ability of N_2O to diffuse out of the soil before it can be further reduced. The slow diffusion rate through the subsoil also results in longer periods of time before denitrified gas is measurable at the soil surface. Another reason for higher $N_2O/(N_2O+N_2)$ ratios in the A horizon is that the nitrification process might have contributed to the N_2O emitted from the A horizon where WFPS was comparatively lower (80%) than that of the two other horizons (85-88%). Aulakh et al. (1996) reported a 100% nitrification of applied ammonium at 80% WFPS within 10 days which declined to 82-90% at 120% WFPS (flooded soil) within 30 days, indicating the sensitivity of just a trace level of O_2 to both nitrification and denitrification. Total organic N, being higher in the A horizon than the two subsoil horizons, can be transformed to nitrate and thus contributed to higher N_2O production by nitrification because the A horizon had comparatively higher (WFPS 80%) aeration than the B and C horizons (WFPS 85-88%). High $N_2O/(N_2O+N_2)$ ratios are the characteristic of fairly well-aerated soils, in which N_2O can easily diffuse away, and thus is not further reduced to N_2 by denitrifying organisms (Webster and Hopkins, 1996). Also the presence of high NO_3^- in top soil can decrease further reduction of N_2O to N_2 (Bandibas, et al., 1994). Schlegel (1992) explained

this phenomenon by stating that NO_3^- is preferred as an electron acceptor with respect to N_2O. The N_2O can also be produced simultaneously by nitrification and denitrification (Khalil and Baggs, 2005), so the production of N_2O from nitrification could affect calculated N_2O to (N_2O+N_2) ratios (Elmi et al., 2005). These factors result in subsoil conditions favouring N_2 as the dominating end product of denitrification. N_2O produced by nitrification is prone to be consumed by denitrification via N_2O uptake and reduction by N_2O reductase activity (Dannenmann et al., 2008). Thus, N_2O and N_2 can be produced simultaneously under adequate supplies of nitrate and C sources in the A horizon. On the other hand, subsoil denitrification could be an important NO_3^- removal pathway to limit nitrate contamination to surface water and groundwater as well as atmospheric build-up of N_2O, provided that there is an available C source to drive the denitrification sequence to completion.

4.4.4 Relationships between potential denitrification rates and their controlling factors

The strong positive relationships of potential denitrification rates with total soil organic C content and not with water-soluble organic C (WSOC) suggests that this fraction is not the only candidate for an electron donor and that the total organic C contains other C sources, which might also influence denitrification. Similarly, Hill and Cardaci (2004) reported a weak and insignificant correlation between WSOC and denitrification potential in mixed and conifer forest soils. Well et al. (2001) found a positive linear relationship between denitrification and total organic C in a shallow groundwater zone. Richards and Webster (1999) and Brettar et al. (2002) also observed a similar relationship in a soil that contained labile C, which was assumed to have been relatively bioavailable. It is likely that the organic C in grassland produced more mineralizable C fractions which are more important than the WSOC (assumed to be equal to DOC) for denitrification to occur. Siemens et al. (2003) revealed that the DOC leached from some agricultural soils contributed negligibly to the denitrification process because the DOC appeared not to be bioavailable. Khalil and Richards (2010), however, postulated that dissolved organic C, oxidation-reduction potential and the substrates (C and N) load differences between the land uses could regulate the degree of denitrification capacity/potential in soils.

Both positive and negative correlations have been reported between soil pH and potential denitrification rates (N_2O, N_2) (Scholefield et al., 1997; Brady and Weil, 2002). The activity of N_2O reductase enzyme is generally thought to increase with increasing pH values (Chapuis-Lardy et al., 2007). Denitrification itself can increase pH by releasing CO_2 and hydroxide (OH^-). However, 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). In our case, the soil was a gleysol with pH values close to 5 in the 1.20-1.30 m soil depth which had lower denitrifier populations than A horizon affecting overall relationships.

The negative correlation between potential denitrification rates and the soil NO_3^- content might be attributed to the reduction of NO_3^- to N_2O and N_2 and/or immobilization to some extent (Scholefield et al., 1997), as the NH_4^+ concentrations at the end were similar to the initial level. Figure 4.2 showed that 3-61% of applied nitrate converted to N_2O+N_2 (TDN) through denitrification, regardless of treatments and soil depths. The NH_4^+-N was positively correlated with denitrification rates, whereas total inorganic N showed a rather weaker and negative correlation. This indicates that NH_4^+ was assimilated into the cells of denitrifiers and enhanced both the denitrifying population and activity (Buss et al., 2005).

The potential denitrification rates (N_2O, N_2 and N_2O+N_2 fluxes) were positively correlated with total N and TON content, the former is in line with the findings of Ciarlo et al. (2007). This indicates that soil total N might have provided adequate amounts of NO_3^- and NH_4^+ to the substrate pool after mineralization. Bandibas et al. (1994) proposed that N_2O emissions were affected by the $N_2O/(N_2O+N_2)$ ratio. Thus, denitrification is a complex process and the soil and environmental factors that influence the process are interrelated. Any variable controlling the N_2O emissions can be a rate-limiting one at different times, depending on particular conditions (Dobbie and Smith, 2003).

There is potential for subsoil denitrification to be enhanced by the introduction of available C sources into subsoils which can be directly or indirectly managed. Fenton et al. (2008) recommended the use of C substrates directly in constructed permeable reactive barriers in subsoils to treat NO_3^- contaminated groundwater, but this is not likely to be cost effective. Manipulation of plant composition and abundance to increase C leaching might indirectly

enhance subsoil denitrification. For example, in arable systems the use of cover crops during the winter recharge has been shown to significantly increase groundwater DOC concentrations (Premrov et al., 2009) and this could also enhance denitrification. In the groundwater beneath soiled water irrigated grassland at JC substantial amount of DOC (25 mg L^{-1}) was measured with nitrate concentration nearly 0.02 mg L^{-1} and the $N_2O/(N_2O+N_2)$ ratio of 0.01 (Chapter 6). This indicates the influence of land use and management, supplying energy sources to denitrifiers, on the reduction of groundwater nitrate and dissolved N_2O. The potential implication of denitrification in subsoil implies that leaching would be decreased through reduction of NO_3^- to N_2O and the N_2O produced would be further reduced to N_2 during diffusional transport into the atmosphere and/or to groundwater.

4.5 Conclusions

The rates of N_2O emission and TDN (N_2O+N_2) under potential conditions were generally greater in the surface soil than in the subsoils, irrespective of the supply of NO_3^- alone or coupled with C sources in the form of glucose and DOC. Addition of C markedly increased soil denitrification rates, giving higher $N_2O/(N_2O+N_2)$ ratios in the surface soil than in the subsoils. This indicates the potential of subsoils for more complete reduction of N_2O to N_2 while the energy sources for denitrifiers are available. Losses of added NO_3^- via denitrification were 25% in A horizon and 3% in C horizon which increased to 45-61% in A horizon and 17-18% in C horizon after C addition. The results suggest that without C addition, potential denitrification rate below the rooting zone was low. Denitrification potentials were mainly regulated by substrates including total organic C, total N and TON. The findings suggest that both glucose-C and DOC were highly effective for the complete reduction of NO_3^- to occur in subsoil environments and subsoils could have a large potential to attenuate NO_3^- that has leached below the rooting zone, with the production of more N_2 than N_2O, if available C is not limiting.

CHAPTER 5. HYDROLOGIC REGIMES OF THE STUDY SITES

5.1 Overview of this chapter

This chapter describes the hydrologic regimes of the study sites and spatio-temporal distributions of hydrochemical properties across sites and vertical profile of groundwater zones. The abundance of NO_3^- in connection to the impacts of site hydrology, groundwater travel time and hydrochemistry is reported. The details of piezometer installation, groundwater sampling and analysis are explained in this chapter.

5.2 Water budget across sites

5.2.1 Background

The spatio-temporal responses of NO_3^- in groundwater to the local hydrology and the delivery of NO_3^- to surface waters are closely linked to the availability and amount of water that percolate through the rooting zone to groundwater, and to surface waters. A catchment scale water balance study was carried out to estimate the amount of water drained out through the rooting zone to the groundwater, termed here as effective rainfall (ER), (Lerner, 1990; Rushton, 2003). This approach is based on the principle that rainfall is input to soil moisture with actual evapotranspiration (AET), soil moisture deficit (SMD) and recharge as the output (Misstear et al., 2008; Misstear et al., 2009). A water balance study can be of use to know the rainfall and effective rainfall pattern in a water year (Bob Zlomke, 2003). The advantages of water balance methods are that they use readily available data, are rapid to apply and account for all water entering the system (Lerner, 1990). The objective of the water balance study was to estimate the effective rainfall and the amount of dissolved C and N (including N_2O, CO_2 and CH_4) lost from groundwater to surface waters over a water year. Water balance also gives insights into the GWT responses to rainfall over the year and the pattern of nutrients flush from top soils and unsaturated zone to the GWT.

5.2.2 Estimating the Water Budget

The general hydrological equation which describes the water balance of the unsaturated zone, as presented by Tindall and Kunkell (1999), was used:

$$P - Q \pm \Delta S_w - E \pm \Delta S_s - D = 0 \qquad \text{(Eqn. 5.1)}$$

where P is precipitation (mm),
Q is runoff (mm),
ΔS_w is change in storage of water ponded on the surface (mm),
E is evapotranspiration (mm),
ΔS_s is change in soil-moisture unrecovered by vegetation (mm),
D is deep percolation unrecoverable by vegetation (mm)

On an annual basis, it can be assumed that change in storage will yield a positive output (Kiely, 1997). However, over a hydrological year (1st October to 30th September), the GWT generally remains at the same level, suggesting the storage is zero. Therefore, the basic method for determining the amount of water available as drainage (leachate) from the ground-surface is to apply a simple balance equation:

Effective Rainfall = Rainfall - Evapotranspiration - Runoff (Eqn. 5.2)

Runoff on all of the farms was deemed negligible because the farm sits on a topographic plateau and the soils are free draining (Bartley, 2003) at OP and DG sites, and higher infiltration capacity than precipitation was measured at other sites (Fenton et al., 2009a). The modified Penman-Monteith equation (Allen et al., 1998) was used to process the potential evapotranspiration (PET), subsequently the hybrid model for computing soil moisture deficit (SMD) described by Schulte et al. (2005) under well to poorly drained conditions to obtain the actual evapotranspiration (AET). Daily weather data were collected from the local weather station situated at the close proximity of each site.

$$PET = \frac{0.408\Delta(R_n - G) + \gamma \dfrac{900}{T+273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \qquad (Eqn. 5.3)$$

where PET is potential evapotranspiration (mm d^{-1}), R_n is the net radiation at the crop surface (MJ $m^{-2}d^{-1}$), G is soil heat flux density (MJ $m^{-2}d^{-1}$), T is air temperature at 2 m height (°C), u_2 is wind speed at 2 m height (m s^{-1}), e_s and e_a are the saturation and actual vapour pressure (kPa $°C^{-1}$), γ is the psychrometric constant (kPa $°C^{-1}$), Δ is the slope vapour pressure (kPa $°C^{-1}$). The principal meteorological input factors determining PET are solar radiation, air temperature, air humidity and wind speed. As soil heat flux G beneath the crop reference surface was relatively small, it was ignored when calculating for day periods. The FAO guide provides a detailed step-by-step approach for all the computations of the required data (Allen et al., 1998).

The AET is a function of the PET and the current SMD, which is calculated as (Schulte et al., 2005):

$SMD_t = SMD_{t-1} - P_t + AET_t + Drain_t$ (Eqn. 5.4)

where SMD_t is soil moisture deficit at day t (mm), SMD_{t-1} is soil moisture deficit at day t-1 (mm), P_t is daily precipitation (mm), AET_t is daily actual evapotranspiration (mm), $Drain_t$ is amount of water drained daily by percolation and/or overland flow (mm) with $Drain_t = -SMD_{t-1}$ (soil moisture > field capacity)

AET_t is calculated as:

$$AET_t = PET \frac{SMD_{\max} - SMD_{t-1}}{SMD_{\max} - SMD_c} \qquad (Eqn.5.5)$$

when $SMD_t > SMD_c$ with SMD_{max} is maximum soil moisture deficit (mm), SMD_c is critical soil moisture deficit (mm). Effective drainage (ED) was calculated by subtracting daily AET from daily rainfall (P) assuming no overland flow. SMD on the day one for each year (1 January, 2009 and 1 January 2010) was set to zero and ER was estimated for each subsequent day. Effective rainfall calculations allow delineation of recharge and non-recharge periods. Calculations were carried out for the study period (Feb 2009 - Jan 2011). The weather data used were recorded in proximity to each study site at Met Éireann weather stations at the research centres.

5.2.3 Groundwater Table (GWT) Fluctuations

Thickness of the unsaturated zone was quantified by measuring changes in GWT depth bgl. The GWT changes were measured continuously for 30 minute intervals over the experimental period using DIVER (Eijkelkamp, The Netherlands). Groundwater depth was compensated with the atmospheric pressure measured using a Baro DIVER (Eijkelkamp, The Netherlands). Monthly measurement of GWT was also carried out manually by electric dip meter before the commencement of sampling. Positions of all wells in context with the elevation of ground surface were carried out using a *Trimble* Global Positioning System (GPS) to determine the elevation of each piezometer wellhead.

5.2.4 Borehole instrumentations across sites

Thirty specifically designed multilevel piezometers (0.05 m ID and 2-6 m screen length: 2 m at JC and SH and 6 m at OP) were installed along groundwater flow paths to target subsoil: 4-6, bedrock-interface: 10-12 and bedrock: 18-30 m bgl at JC, SH and OP and 6 single wells in only bedrock (30-50 m bgl; 6 m screen section) at DG. The piezometers were installed based on the local hydrological conditions to sample groundwater along its flow paths. Positions of all wells in context with elevation were carried out using a *Trimble* Global Positioning System (GPS). The details of the ground level elevation for each well, piezometer screen position and GWT are shown in Figure 5.1, 5.2, 5.3 and 5.4 for JC, SH, OP and DG, respectively. An example of the instrumentation of a multilevel well is shown in Figure 5.5. A soil exploration drilling rig (Giddings drill) was used to install piezometers where water struck above bedrock, whereas a rotary air drilling was used to drill where it struck within the bedrock. The materials used at different layers in the borehole are illustrated in Figure 5.5. Well development was carried out by pumping the wells for several times over two months after installation as long as groundwater was clear using a centrifugal pump (Model MP1, Grundfos, Fresno, CA, USA). Well integrity was checked for each borehole by adding water to increase water height to 1 m above the static level in one well and measuring changes in height in adjacent two wells using DIVER (Eijkelkamp, The Netherland).

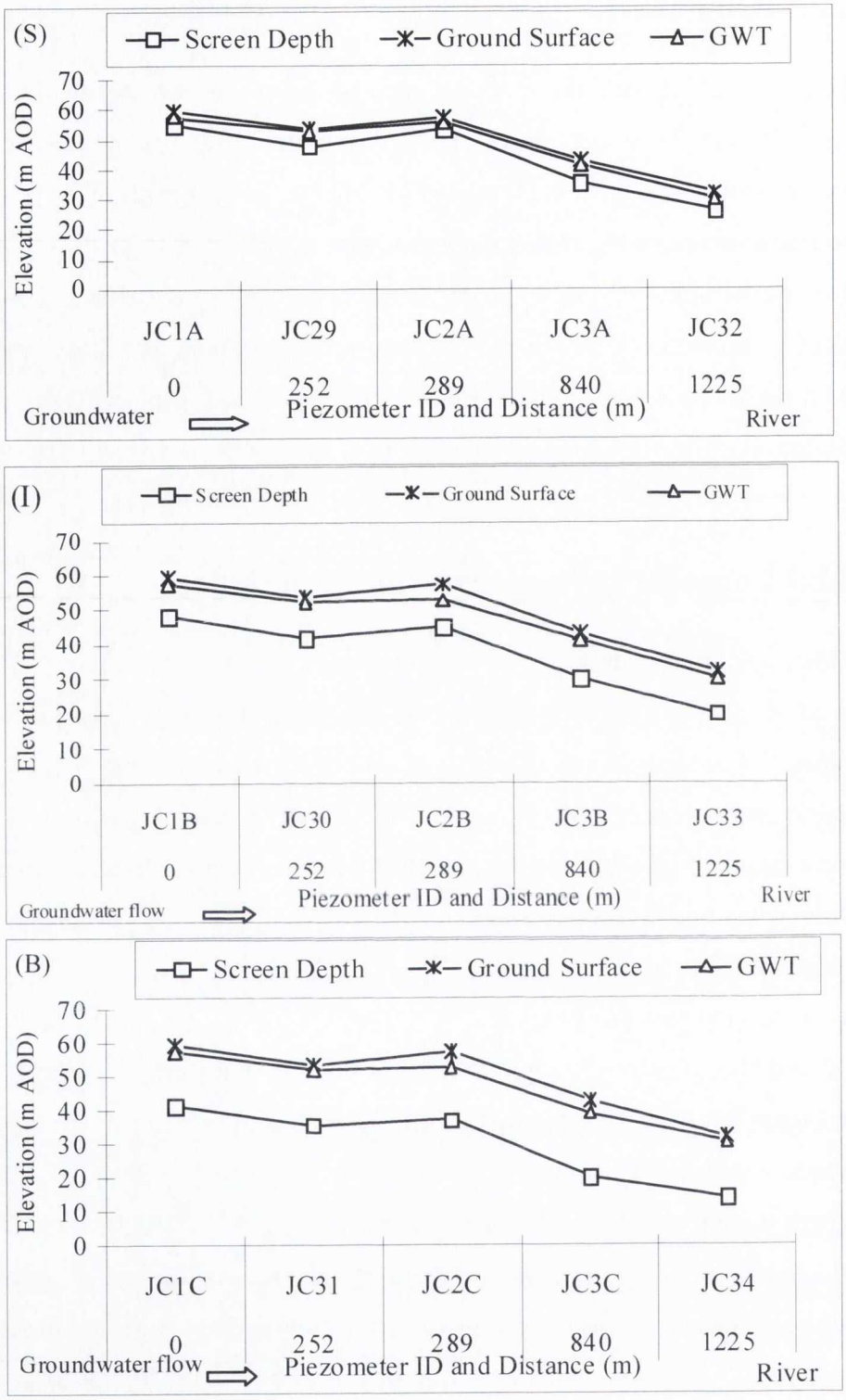


Figure 5.1 Land topography, piezometers' positions and groundwater table (GWT) depth (m AOD) at Johnstown Castle in (S), subsoil; (I), bedrock-interface and (B) bedrock

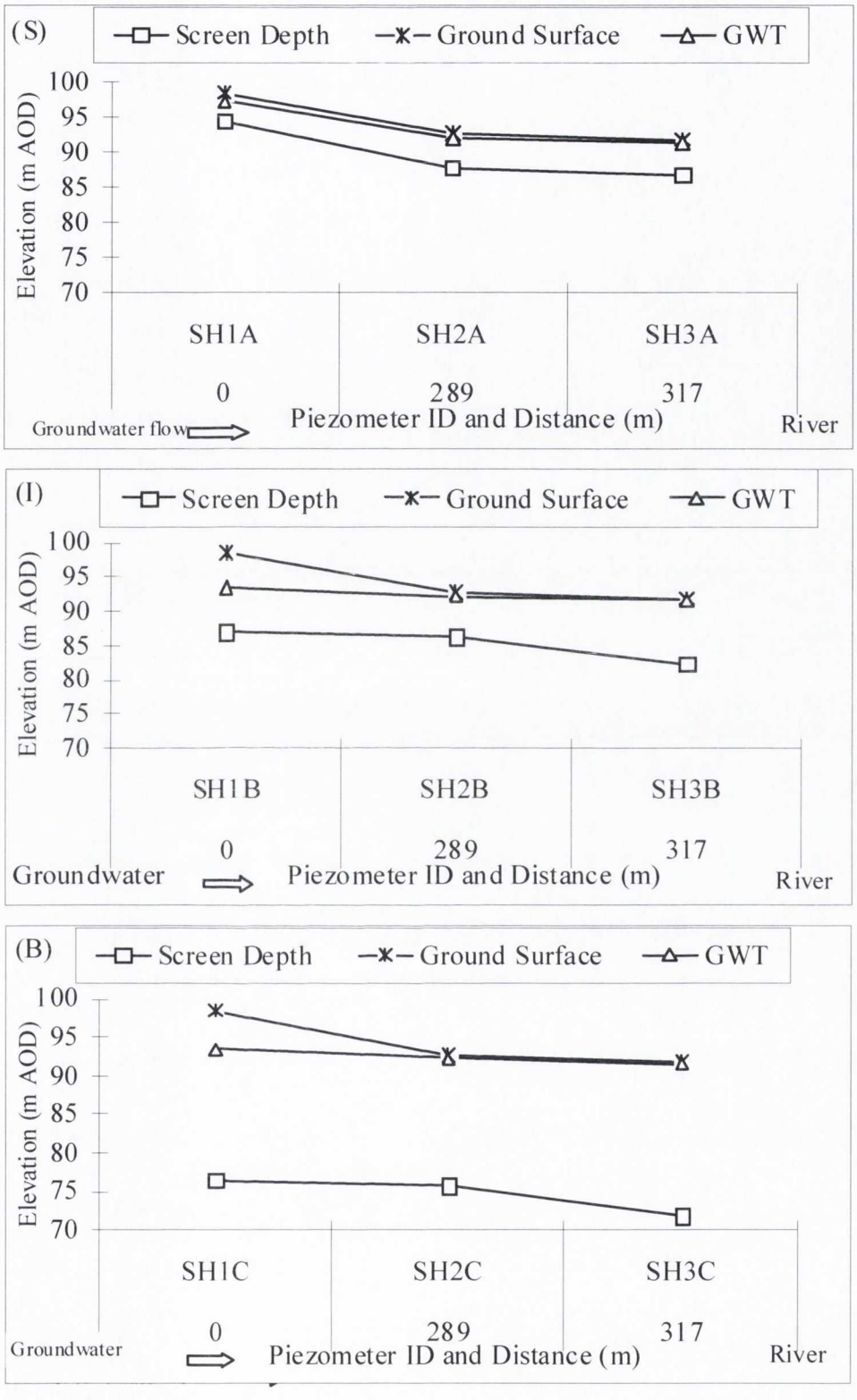


Figure 5.2 Land topography, piezometers' positions and groundwater table depth (m AOD) at Solohead in (S), subsoil; (I), bedrock-interface and (B) bedrock

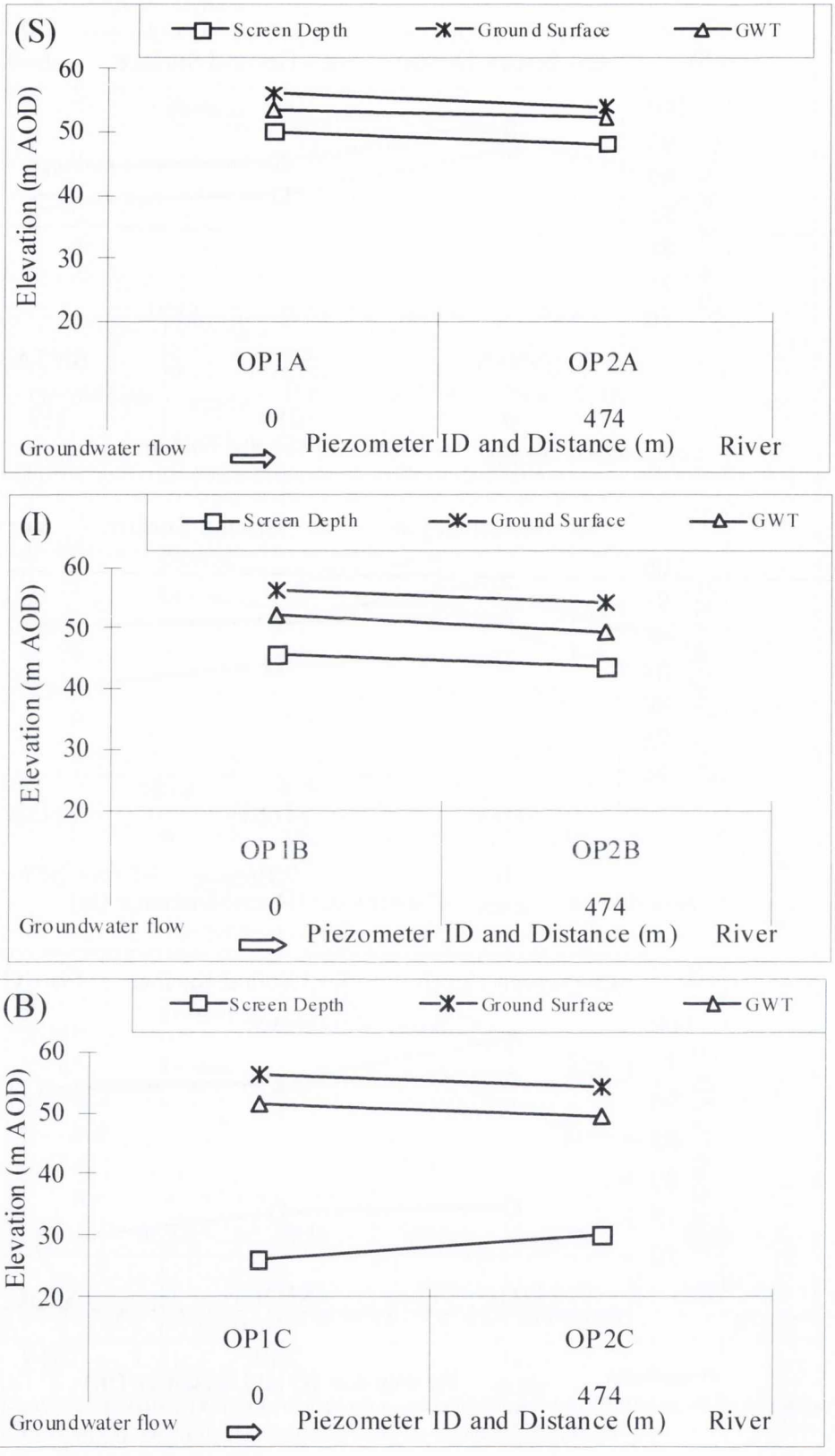


Figure 5.3 Land topography, piezometers' position and groundwater table depth (m AOD) at Oak Park (S), subsoil; (I), interface and (B) bedrock

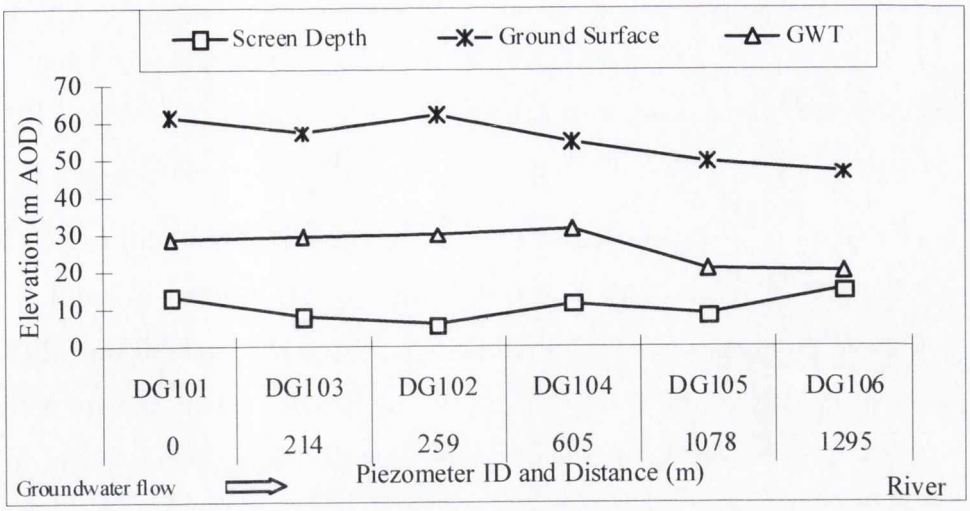


Figure 5.4 Land topography, piezometers' position and groundwater table depth (m AOD) at Dairy Gold (S), subsoil; (I), interface and (B) bedrock

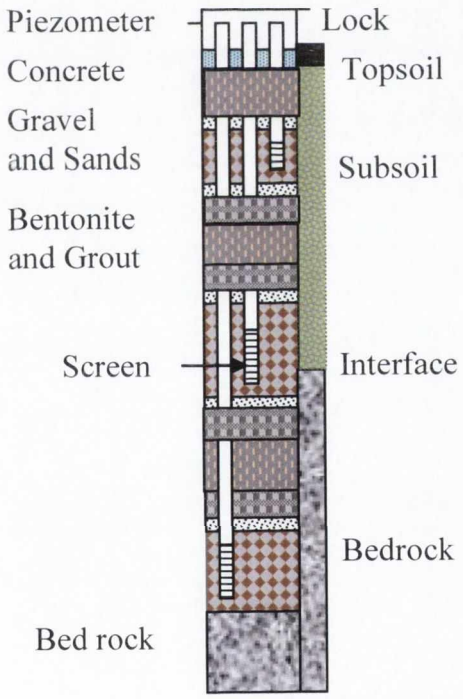


Figure 5.5 A sketch showing the instrumentation of bore hole and installation of multilevel piezometers

5.2.5 Results

5.2.5.1 Total and daily rainfall

Different study sites showed different hydrologic regimes with respect to the amount of rainfall recorded over the two years. Though the patterns of rainfall over times were similar in both years, 2009 showed significantly higher rainfall than 2010 (Table 5.1) that created marked contrast in unsaturated zone water content and its delivery to GWT. In general, the highest rainfall among the four different sites was recorded at JC (mean rainfall 1200 mm y^{-1}) and the lowest at OP (mean rainfall 963 mm y^{-1}) but JC and SH sites were comparatively wetter than OP and DG sites (Table 5.1). Rainfall in Ireland showed an increasing trend over the last couple of years except 2010. The rainfall in 2009 was 30-40% above the average and in 2010 it was 10-15% below the average. The highest amount of total rainfall occurred during October to December in each year at each site but was reverse during July to September (Figure 5.6a, 5.7a, 5.8a and 5.9a).

Table 5.1 Annual rainfall, potential evapotranspiration (PET), actual evapotranspiration (AET) and effective rainfall (ER) data from 2009 to 2010

Hydrologic events	JC		SH		OP		DG	
	2009	2010	2009	2010	2009	2010	2009	2010
‡P (mm)	1452	947	1403	879	1167	759	1293	869
Mean P for 2009-2010 (mm)	1200		1141		963		1081	
Mean P for 1999-08 (mm)	1053		1004		890		998	
‡PET (mm)	632	633	681	686	713	718	694	700
‡AET (mm)	615	562	643	553	630	518	620	543
‡ER (mm)	836	385	759	326	537	241	673	326
Mean for 2009-2010 (mm)	611		543		389		500	
No. of days ER (d) occurred	211	168	200	45	83	43	105	50
Portion of P as ER (%)	57	41	54	41	46	32	52	38

‡P: precipitation, PET: potential evapotranspiration, AET: actual evapotranspiration and ER: effective rainfall

5.2.5.2 Total and daily effective rainfall (ER)

Both the PET and AET were quantified as approximately similar between the study sites in each year. However, the ER was different between sites, being highest at JC and lowest at OP (Table 5.1). The portions of total rainfall that became ER were 57 and 41, 54 and 41,

46 and 32, and 52 and 38% between 2009 and 2010 respectively, at JC, SH, OP and DG. The highest ER was observed during October to December and the lowest during July to September at all sites (Figure 5.6b, 5.7b, 5.8b and 5.9b). An abrupt increase in cumulative ER was observed during October to December is shown in Figure 5.5. Moreover, a longer period of ER occurred at JC and SH sites than at OP and DG (Table 5.1).

5.2.5.3 Groundwater table fluctuations (GWT)

Groundwater table fluctuations followed the patterns of rainfall and ER over time (Figure 5.6c, 5.7c, 5.8c and 5.9c). The shallowest GWT was 0.6, 0.7, 2.0 and 24.7 m bgl, measured during October to December respectively, at JC, SH, OP and DG, whereas the deepest GWT was 2.5, 1.4, 5.5 and 30.5 m bgl, measured during July to September in all depths and sites (Figure 5.6c, 5.7c, 5.8c and 5.9c). Therefore, the annual GWT fluctuations were within 1.9, 0.7, 3.5 and 5.3 m. The GWT was different in subsoil, interface and bedrock with shallowest in subsoil and deepest in bedrock at all sites except at DG where there was no water in subsoil and interface (Figure 5.6c, 5.7c, 5.8c and 5.9c). Mean GWT was significantly deeper at OP and DG than at JC and SH ($p < 0.05$). Mean GWT over the two years ranged from 1.7 m bgl in subsoil to 2.8 m bgl in bedrock at JC; 1.0 m bgl in subsoil to 2.1 m bgl at SH; 3.0 m bgl in subsoil to 5.4 m bgl in bedrock at OP and was 29 m bgl at DG (Table 5.2). A high resolution GWT changes data were recorded in every 30 min interval and presented in Appendix 12. The depth of unsaturated zone (USZ) at the four study sites, as revealed from the depth of GWT, indicated the contrasting hydrogeological conditions of the sites.

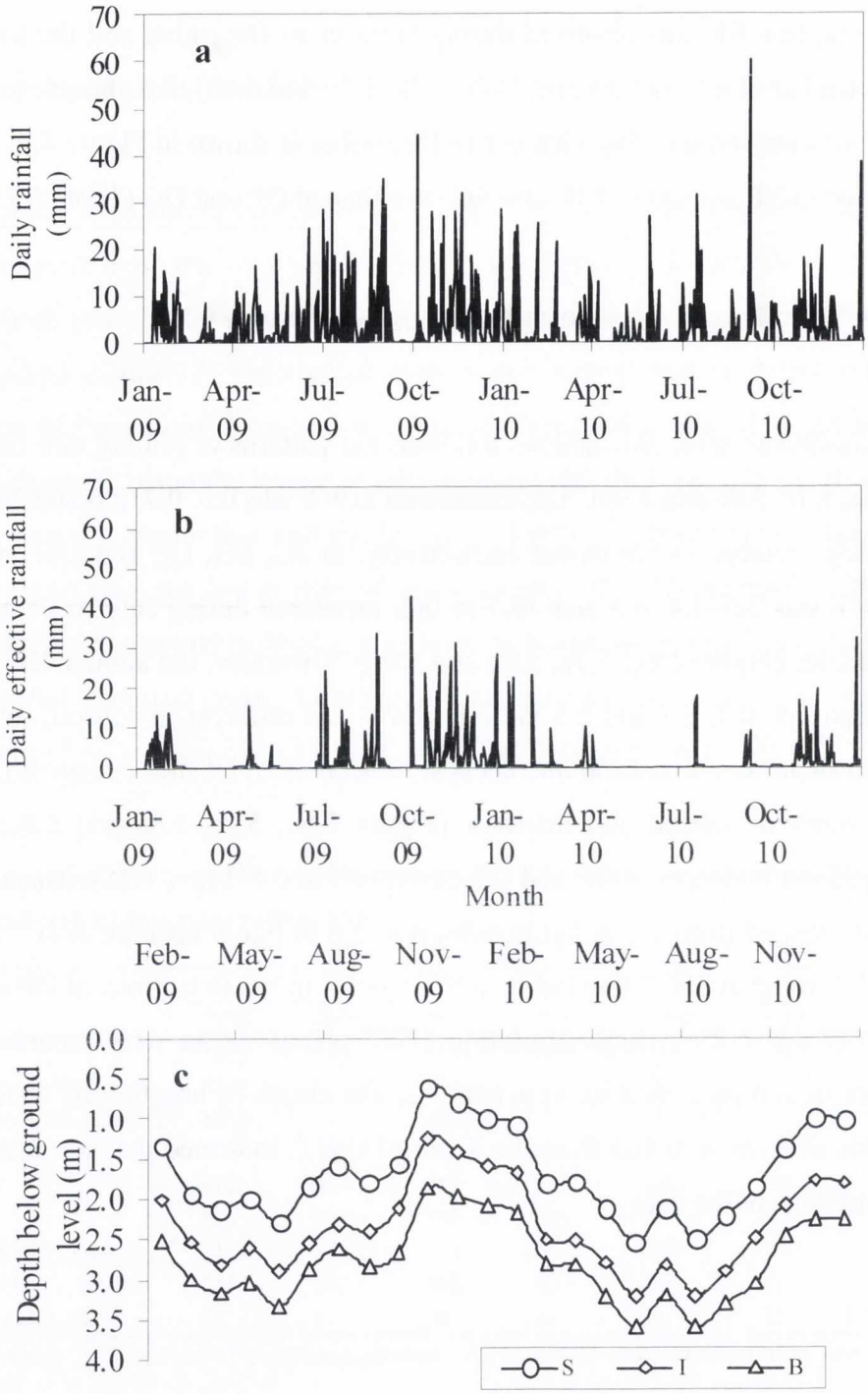


Figure 5.6 Monthly (a) rainfall, (b) effective rainfall and (c) GWT fluctuations in (S) subsoil, (I) Interface and (B) Bedrock at JC from 2009-2010.

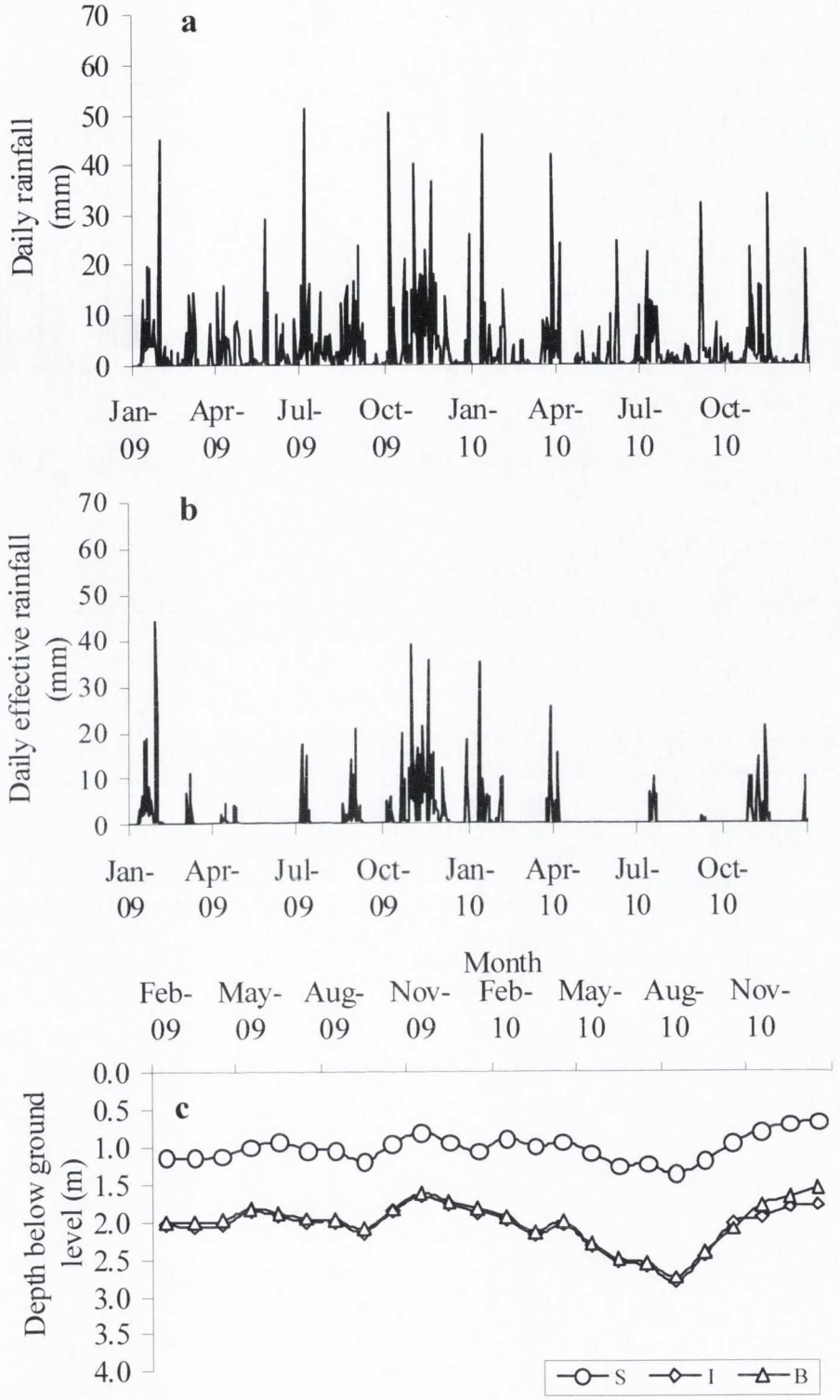


Figure 5.7 Monthly (a) rainfall, (b) effective rainfall and (c) GWT fluctuations in (S) subsoil, (I) Interface and (B) Bedrock at SH from 2009-2010.

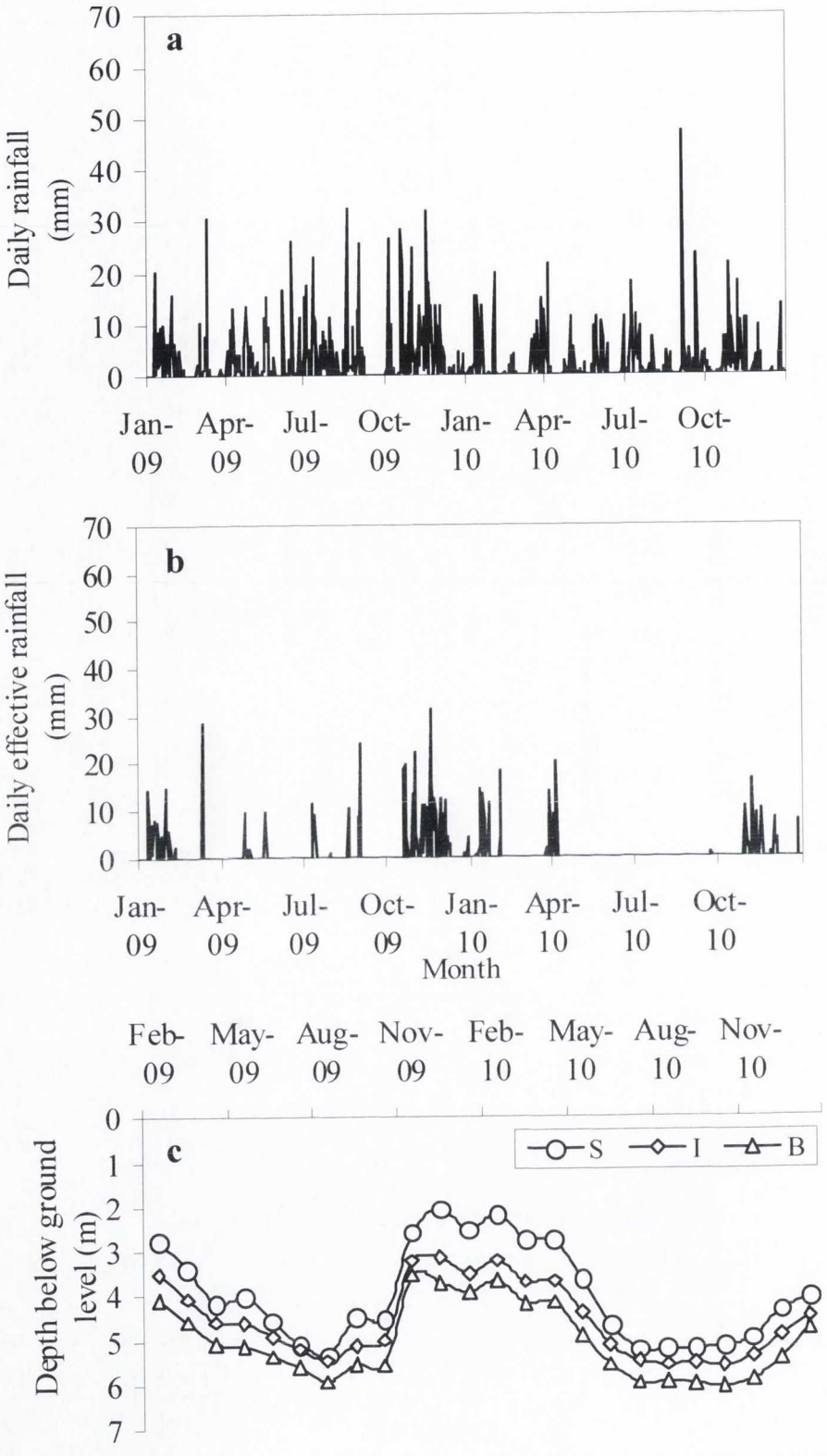


Figure 5.8 Monthly (a) rainfall, (b) effective rainfall and (c) GWT fluctuations in (S) subsoil, (I) Interface and (B) Bedrock at OP from 2009-2010

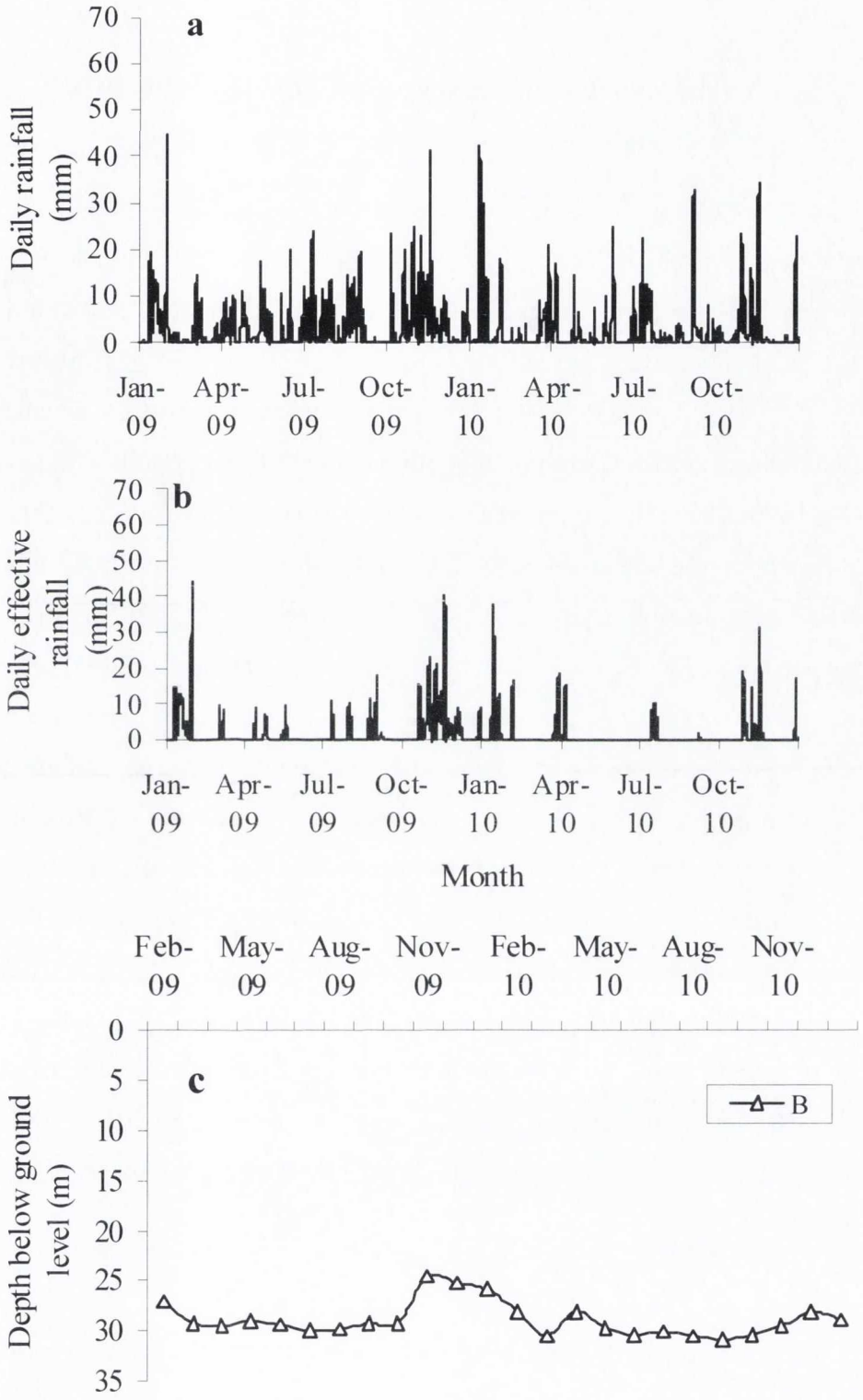


Figure 5.9 Monthly (a) rainfall, (b) effective rainfall and (c) GWT fluctuations in (S) subsoil, (I) Interface and (B) Bedrock at DG from 2009-2010

5.2.6 Discussion

5.2.6.1 Variations in rainfall across sites during 2009-2010

The average rainfall during the study period was comparatively higher than the preceding ten years (1999-2008), e.g. 1053, 1004, 890 and 998 mm at JC, SH, OP and DG, respectively. Despite the two contrasting years of rainfall, mean rainfall was within the range of mean Irish rainfall (800-1400 mm) (Daly, 1995). However, an index of total annual precipitation for Ireland, based on averaging 11 of 14 weather stations, shows a general trend of increasing precipitation over a 40 year period, with notable increases since the 1970s (EPA, 2008). At the JC site, total rainfall in 2006 and 2007 was 993 and 889 mm, respectively (Fenton et al., 2009a) which were 1452 and 947 mm in 2009 and 2010. Mean precipitation during the 2005–2008 periods at JC and SH was 1046 and 1059 mm, respectively (Fenton et al., 2009b). However, Schulte et al. (2005) reported 20 years (1985 to 2005) average rainfall at JC as 1034 mm. The JC and SH sites are comparatively wetter than the OP and DG. Daly (1995) noted that the AET in Irish conditions ranged from 400-500 mm and therefore the excess of rainfall over AET ranged from 400-1000 mm. Mean precipitation during the 2005–2008 period was 858 and 953 mm, respectively at OP and DG sites (Fenton et al., 2009b) which are in well agreement with the present estimation. The total rainfall, AET and the ER near the vicinity of DG sites were measured during 1993-1995 by Richards (1999); 2000-2002 by Bartley (2003) and were comparable to the results of the current study with 2009, but were comparatively higher than 2010, indicating that 2010 was a dryer year than the preceding years in Ireland. Therefore, it appeared that in 2010 dissolved N including NO_3^- flushed out from the soil and subsoils to groundwater was low.

5.2.6.2 Groundwater table (GWT) response to effective rainfall (ER)

The response of GWT to rainfall depends on the amount of rainfall and the evaporative demand plus the SMD. The ER between 2009 and 2010 was different at all sites. However, ER at JC and SH appeared to have occurred for a longer period than OP and DG sites showing a longer period for rainwater recharge at JC and SH sites. Fenton et al. (2011)

reported the ER of 600 mm for JC site which is comparable to the present estimations (385-836 mm). However, mean ER for the JC site during 2005-2008 was 535 mm and occurred on 178 days (Fenton et al., 2009b). Mean ER at the SH site during 2005-2008 was 481 mm and occurred on 91 days (Fenton et al., 2009b), which is comparable to the present estimation (Table 5.1). The ER value at OP site is comparable with the ER measured by Walmsley (2009) of 524 mm in 2007 and of 559 mm in 2008. Premrov (2011) calculated the percentage of total rainfall that became ER at OP as 41 and 43%, respectively in 2007 and 2008, which is within the range of the present estimation (32-46%). The estimated ER at DG site is in good agreement with Landig (2009) who calculated the ER of 426 and 568 mm in 2007 and 2008, respectively which accounts for 47 and 54% of the total rainfall. Bartley (2003) estimated the ER of 679, 644 and 537 mm which accounts for 58, 65 and 51% of the total rainfall in 2000, 2001 and 2002, respectively at a Farm near the vicinity of DG, and is in good agreement with the present estimation. At DG, Fenton et al. (2009b) estimated mean effective rainfall during 2005-2008 of 437 mm to have occurred on 159 days. Therefore, a higher portion of ER over the total rainfall at JC and SH sites (41-57%) than OP and DG sites (32-52%) could be due to the longer period of ER as well as the shallow USZ in the former sites. Because the shallow USZ is consistent with low permeability and eventually with lower SMD than the high USZ and thus increases ER.

During July to September evaporative demand plus soil moisture deficit exceeded the amount of rainfall resulting in a drop in GWT. The GWT rise pattern results are comparable with the report of Bartley and Johnston (2006), wherein they observed groundwater recharge following the ER begins in October of each year and continues until May or June. In general, there is a recession period for ER during March to September in each year at all sites. Therefore, a steady decreasing trend in GWT during this 7 month period was observed every year in all sites. When the ER events occurs a GWT rise take place almost instantaneously (Bartley and Johnston, 2006). Bartley (2003) observed highest ER during November to February in a study in a Farm during 2000-2003 which was comparable with the present results. Daly (1995) noted that there is a definite recharge period from mid-October to mid-March and thereafter, a general recession period of up to 7 months occurs. During this dry period, the possibility of nutrient leaching to groundwater is declined.

The depth of USZ, as delineated by the depth of GWT bgl, and the annual fluctuations in GWT were lower at JC and SH sites than OP and DG sites which are indicating lower permeability of groundwater at JC and SH than at OP and DG. Annual fluctuations of GWT ranged from 0.7 m at SH to 5.3 m at DG which increased with the increase in the permeability of aquifer, being the highest at DG and lowest at SH. Fitzsimons and Misstear (2006) noted that annual GWT fluctuations in Irish conditions are within 5 m, which is comparable with these study sites. Past results at JC site showed that mean GWT depth in subsoil with K_{sat} values ranging from 0.007-0.016 m d^{-1} was 2.2 m bgl (Fenton et al., 2009a). An approximately similar depth to GWT was observed at the beginning of the hydrological year that starts on 1 October and at the end of the hydrological year on 30 September. This suggests that the ER is an approximate amount of groundwater that is discharged to the receptors during a hydrological year. Fenton et al. (2009) suggested that all of the ER reached the GWT as the rainfall intensity is generally lower than the soil infiltration capacity in the same study area. Bartley (2003) defined potential recharge as the amount of rainfall that exceeded the amount of AET. In addition, runoff losses and lateral flows of rainfall can be assumed to be zero in the agricultural catchments in Ireland (Bartley, 2003; Fitzsimons and Misstear, 2006). The interflow can be negligible in the research areas e.g., because of shallow GWT during the period when ER occurs at JC and SH sites and because of free draining characteristics of soils and tills at OP and DG sites which facilitate rainwater infiltration vertically to the groundwater. However, if there is any pathway that can prevent ER from reaching groundwater can be compensated by the contribution of groundwater from deeper bedrock zone to the groundwater under study (5-50 m bgl), as confined nature of groundwater especially in interface and bedrock was exerted by their different piezometric heads/potentiometric levels from the subsoil. In addition, groundwater from the deeper bedrock out side the screen depths can contribute to the receptors due to its nature of flow (curvilinear to water table) from groundwater to the river, which can also compensate surface water loss or loss by seepage through USZ. Therefore, the total amount of ER occurred at each site can be considered as the amount of groundwater discharged to the receptors e.g., river in the study area, because the change in groundwater storage Irish conditions is zero (Fitzsimons and Misstear, 2006). The period of ER is very important for agricultural management practices as it contributes to the delivery of nutrients e.g., NO_3^- to groundwater. The year 2010, in compare to 2009, showed a lower NO_3^- delivery to groundwater which might reduce NO_3^- feeding to rivers

in the subsequent years. The hydrogeochemistry of groundwater is influenced by ER mainly because it controls the delivery of DO and DOC and other nutrients from top and subsoils to the GWT e.g., low DO recharge to groundwater during the recession period of ER can increase NO_3^- reduction by denitrification. The rainwater and afterward the ER while recharging the groundwater equilibrate with DO and thus enhance the DO concentrations in groundwater (Rivett et al., 2008). One important hydrologic insight here is that higher hydraulic loading increases the higher volume of groundwater delivery to the river. Because at JC, SH, OP and DG sites 485, 393, 296 and 348 mm more ER were estimated in 2009 than in 2010, but the mean GWT rise in 2009 over 2010 was negligible in compare to the amount of ER, being 0.22, 0.16, 0.06 and 1.16 m, respectively.

5.3 Measuring hydraulic conductivity across the study sites

5.3.1 Background

Hydraulic conductivity is the most important soil property that controls water and solute movement in soils. It is an important aquifer parameter that indicates the ability of sediments to transmit the water (Fetter, 2001). Groundwater hydraulic conductivity is important mainly to estimate the recharge and vulnerability of groundwater and eventually to know the hydrogeochemical processes. In groundwater, in order to determine the hydraulic properties of aquifer material e.g. the ability of aquifer material to transmit water through the aquifer can be determined by a large-scale pumping test (Theis, 1935; Cooper and Jacob, 1946). In unconfined aquifer considering two or three dimensional flow of unconfined units, a large-scale pumping test can not be representative but a hydraulic conductivity (K_{sat}) can be measured by performing a single well slug test (Freeze and Cherry, 1979). A slug test is the test in which water level recovery over time is monitored in the well that has had an instantaneous addition or abstraction of water in order to provide an estimate of K_{sat}. During slug test the water level in the borehole is instantaneously dropped (rising head) or raised (falling head) (Misstear et al., 2006). The slug test is simpler and quicker than a conventional aquifer test and works with a relatively small diameter well (Schwartz and Zhang, 2003). In addition, slug tests have become a primary method for analyzing aquifer transmissivity due to their relative speed and

simplicity as compared to more laborious tests such as pumping tests or hydraulic tomography (Cardiff et al., 2011). In the present study areas pumping test was not feasible as all wells had a smaller diameter than the required diameter of pumping test.

Hvorslev (1951) pioneered a methodology for the analysis of a slug test's field data using drawdown of head over time. Bouwer and Rice (1976) determined K_{sat} in a fully or partially penetrated well which is similar to Hvorslev, but requires to use a set of curves to estimate the radius of influence (Schwartz and Zang, 2003). Moreover, this method can be used in both unconfined and confined or stratified aquifers with any diameter and depth of borehole (Bouwer, 1989). This method is based on Thiem's equation for flow into a well after the sudden removal of a slug of water (Kruseman and de Ridder, 1990). Campbell et al. (1990) compared several methods to estimate in situ hydraulic conductivity and concluded that Bouwer and Rice method is the method of choice for slug test because the method's results are consistent with other more cumbersome and time-consuming methods.

5.3.2 Hydraulic Conductivity Measurement

In this study, the Bouwer and Rice (1976) method was used as outlined by ILRI (1990) to estimate saturated hydraulic conductivity of the wells across sites (Fenton et al., 2009b). A slug of water was quickly added to the borehole and the subsequent rate of fall of the water level in the hole was measured. This was possible because the equilibrium water level was above the screened section. The procedure was as follows:

1. The original head in the well is recorded first
2. For the slug injection, the required quantity (to develop considerable water head above the original head e.g. 10 to 50 cm) of water- called a slug is instantaneously added to the borehole. The height of the slug above its original level is termed as t_o (height at time zero).
3. The raised water head will now start to dropdown over time. The change in head is noted over time where *ht* is termed as the height of head at any given time (height at a given time t).

4. The field data were plotted on a semi-logarithmic paper with h_o/h_t on a log-scale (y-axis) against the corresponding time t on the arithmetic scale (x-axis).
5. A straight line was fitted through the plotted points.

The response of well water level over time was measured using an electronic datalogger, 'DIVER' (Eijkelkamp, The Netherlands), set to record at 1-sec time intervals hanging into the well at the middle of the screen section (Figure 5.10). It measures both atmospheric and water column pressures on the optical part of the DIVER in the well. The barometric 'DIVER' was hung in the well keeping it above the water level to measure the atmospheric pressure. Software called 'ENVIROMON' and a laptop computer provided instant access to the datalogger. On return to the laboratory, data were exported to EXCEL for manipulation, plotting and k_{sat} determination. In most cases, the slug test was replicated twice. Changes in water head is plotted against changes in time on a semi-logarithmic paper. In a few wells, anomalies were observed as the plotted head-drawdown yielded a curve with two straight line segments (Figure 5.11). This phenomenon was first explained by Bouwer (1989) as "double straight line effect". The first steeper line segment, indicated with a dotted line, can be attributed to drainage of the gavel pack or developed zone around the well. In this case, the second straight line portion, indicated by solid line, is used to estimate K_{sat} to eliminate such effect and the early data points were ignored.

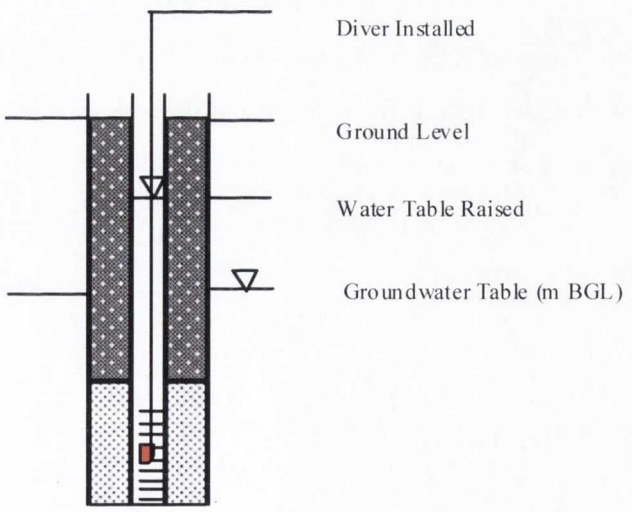


Figure 5.10 Addition of a slug of water and increasing the static water level to a certain height and measuring water table change by an electronic diver

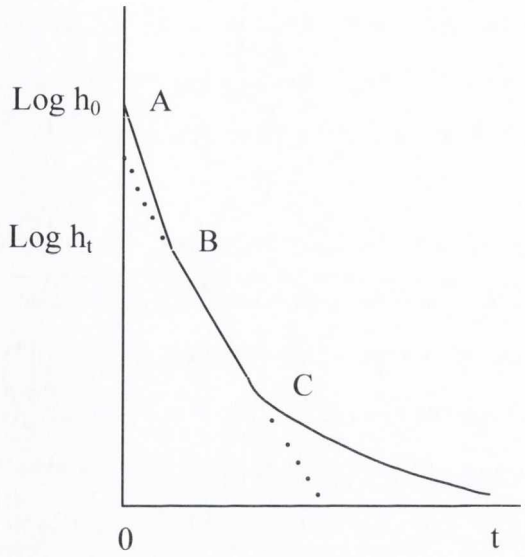


Figure 5.11Schematic of double straight line effect; the first part (AB) is due to drainage of gravel pack and second part (BC) is used to calculate K_{sat} (after Bouwer, 1989)

Calculation of K_{sat}

Data were analyzed after Bouwer and Rice (1976) method for unconfined aquifers in steady-state flow conditions.

$$K_{sat} = \frac{r_c^2 \ln(\frac{R_e}{r_w})}{2d} \frac{1}{t} \ln \frac{h_o}{h_t} \qquad (Eqn.\ 5.6)$$

where r_c is the radius of the unscreened part of the well where the head is rising, r_w is the horizontal distance from the well centre to the undisturbed aquifer, R_e is the radial distance over which the difference in head, h_0, is dissipated in the flow system of the aquifer, d is the length of the well screen, h_0 is the head in the well at time t_0, h_t is the head in the well at time $t > t_0$. As the wells were partially penetrated, the following equation was used for calculating $\ln \frac{R_e}{r_w}$:

$$\ln \frac{R_e}{r_w} = \left[\frac{1.1}{\ln(\frac{b}{r_w})} + \frac{A + B \ln\left[\frac{(D-b)}{r_w}\right]^{-1}}{\frac{d}{r_w}} \right] \qquad (Eqn.\ 5.7)$$

where b is the distance from the water table height to the bottom of the well, D is the distance from the water table to the impermeable zone, A and B are dimensionless

parameters which are functions of d/r_w. If D > > b, the effective upper limit of ln $[D-b)/r_w]$ may be set to 6.

Assumptions for K_{sat} measurement

Bouwer and Rice (1976) method was applicable under the following conditions:

1. The aquifer is homogenous, isotropic, and of uniform thickness over the area influenced by the slug test;
2. Prior to the test, the water table is horizontal over the area influenced by the slug test; the slug of water added instantaneously added to the bore hole;
3. The inertia of the water column in the well and the linear and non-linear well losses are negligible; the well is either partially or fully penetrated the saturated thickness of the aquifer;
4. The well diameter is finite; hence storage in the well can not be neglected.

5.3.3 Results of hydraulic conductivity estimated across sites and depths

The weighted mean values of saturated hydraulic conductivity were $2.0x10^{-2}$, $2.5x10^{-2}$, $7.0x10^{-2}$ and $2.6x10^{-1}$ m d^{-1} respectively, at JC, SH, OP and DG. Saturated hydraulic conductivity in subsoil was significantly higher at OP (K_{sat} $3.3x10^{-2}$ m d^{-1}) than JC (K_{sat} $7.0x10^{-3}$ m d^{-1}) and SH (K_{sat} $5.0x10^{-3}$ m d^{-1}) ($p<0.05$), while the later two were similar (Figure 5.12). At the interface zone, it was also significantly higher at OP (K_{sat} $6.3x10^{-2}$ m d^{-1}) than JC (K_{sat} $2.4x10^{-2}$ m d^{-1}) and SH (K_{sat} $3.6x10^{-2}$ m d^{-1}). In bedrock, hydraulic conductivity was significantly higher at OP and DG ($p<0.001$) than JC and SH but when compared with OP, the DG site showed higher values ($p<0.05$) of hydraulic conductivity than OP. Considering inter depths differences, no significant difference was observed between depths at JC, but at SH subsoil showed significantly lower K_{sat} value than interface ($p<0.01$) and bedrock ($p<0.01$) where latter two were similar. At the OP, hydraulic conductivity was significantly higher in bedrock than in subsoil and at interface. Hydraulic conductivity data were log-normally distributed at JC, SH and DG sites, but at OP they were normally distributed. Spatial variability of groundwater hydraulic conductivity was remarkably higher showing mean coefficients of variation of 61-203, 49-148, 27-57 and 94%.

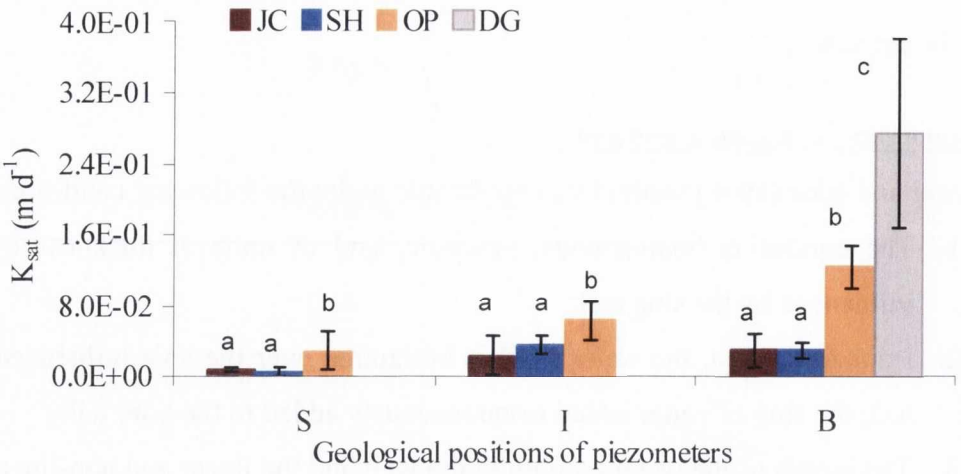


Figure 5.12 Mean (±SE) of saturated hydraulic conductivity at (JC), Johnstown Castle; (SH), Solohead; and (OP), Oak Park in (S), subsoil; (I) bedrock-interface; and (B) bedrock and at DG only in (B), bedrock. The same letter within each depth does not differ significantly between sites (p>0.05)

5.3.4 Discussion

Saturated hydraulic conductivity is an approximation of well permeability which provides important information about the water, dissolved nutrients and gases flow rates in aquifer. Range of hydraulic conductivity values across sites showed approximately two orders of magnitude but within the same site the variation was lower (showing one order of magnitude). Fenton et al. (2009a) measured saturated hydraulic conductivity in 17 wells in subsoil at JC by slug (after the Bouwer and Rice (1976) method) which ranged from 0.001 to 0.016 m d^{-1}. These hydraulic conductivity values were comparable with the range of present study in subsoil. A tracer study carried out by Hooker (2005) in the subsoil zone at OP showed that vertical travel time of tracer (Bromide, Br^-) in unsaturated zone ranged from 0.01 to 0.02 m d^{-1} which is comparable to the saturated hydraulic conductivity in the subsoil of 0.033 m d^{-1}in the present slug test result. At interface and bedrock, the measured hydraulic conductivity values were similar to subsoils at all sites, indicating that groundwater flow rate is approximately uniform across depths of groundwater. Orr et al. (2010) investigated hydraulic conductivity in three different sites in Ireland in subsoils to up to 115 m bgl in bedrock and stated that contaminants may flow equally easily to depth of groundwater. However, higher hydraulic conductivity at OP and DG sites than at JC and SH could be due to the comparatively coarse subsoil type (sands intermixed with gravel)

and high permeable bedrock (limestones). At OP site, an investigation carried out by Fenton et al., (2009b) showed that sand and gravel aquifer with solely intergranular permeability have low travel time of groundwater. Premrov (2011) reported one order of magnitude higher (ranged 0.49-3.37 m d^{-1}) saturated hydraulic conductivity of subsoil (<4 m bgl) measured in February 2008 using Hvorslev (1951) method. This discrepancy could be due to the measurement time i.e., in rainy season. In these same wells, Minerex Environmental Ltd. (Minerex, 2002; unpublished data) performed slug test and observed a range of 0.02-0.53 m d^{-1} which were quite similar to the results of the present study. At Curtin's farm with subsoil overlying karstified limestones near DG site, Bartley (2003) reported saturated hydraulic conductivity in subsoils ranged from 0.004 to 27 m d^{-1}. These results (Bartley, 2003) are higher than the hydraulic conductivity results determined in the present study may be due to the karstification in subsoils. At JC and SH, subsoil was silt clay to heavy clay intermixed with dense gravel and Ordovician sediments of sandstones and shales at JC and Devonian sandstones and mudstones at SH which might have reduced their permeability. Fitzsimons and Misstear (2006) reported the hydraulic conductivity values of some low to moderate permeable tills in Ireland ranging from 0.0004 to 0.009 m d^{-1} which was within the range of the hydraulic conductivity data of the current study at JC and SH sites, being considered as low permeable areas. Lower permeability and high travel time of groundwater due to the fine loamy till in subsoils at JC and SH was also in line with the findings of Fenton et al (2009b). Swartz et al. (2003) suggested that the boundary between low and moderate permeability subsoils appears to be in the region of 10^{-9} m s^{-1} which is comparable to the range of these results across sites. Spatial variability in hydraulic conductivity reflects the spatial heterogeneity of aquifer materials. At JC, higher spatial variability occurred due to the heterogeneity in subsoil texture e.g. a sandy plot on top of the catchment at JC site has higher permeability than other plots (Fenton et al., 2009a). At low permeable sites (JC and SH), generally wells in top of the field have higher permeability than wells in down slope of the field. This can occur due to the removal of clay particles from top slope to the down slope of the filed by leaching and runoff. A wide range of hydraulic conductivity data 0.0048-5.50 m d^{-1}, with a CV value of 102% were reported by Duffera et al. (2007), which they measured in soils and sediment cores collected from the field with loamy sand type soil in Southeastern coastal plain, USA. Higher CV values of hydraulic conductivity at low permeable sites (JC and SH) clearly indicating that low permeable soils and sediments are likely to be spatially more complex

and heterogeneous than high permeable sites (OP and DG), may be due to the higher residence time of nutrients that have longer time to undergo biogeochemical transformations.

5.4 Groundwater flow direction across all catchments

Knowledge of groundwater flow is important mainly due to its implications to identify the recharge and discharge area in an intensively managed agricultural catchment and to understand the risk of pollutant delivery to the stream. Based on the flow pattern of the aquifer system, N inputs in the land and its possible contributions towards the stream can be understood. From the land surface, the water moves to the water table and then it travels and seeps through soil and rock underground. The water table is not flat as its name implies. It is the top of the water surface in the saturated part of an aquifer. Groundwater usually flows from high potential to the low potential, and eventually drains into stream, lakes, rivers, and sea. The flow of groundwater in an aquifer does not always reflect the flow of water on the surface. It is therefore necessary to know the direction of groundwater flow and take steps to ensure that land use activities in the recharge area will not pose a threat to the quality of the groundwater (Freeze and Cherry, 2002).

5.4.1 Determination of Groundwater Flow Direction

Understanding of groundwater flow direction is required to evaluate solute transport along groundwater flow paths. Gravity is the dominating driving force of groundwater and water flows from high elevation to low elevation and from high pressure to low pressure. Gradients in potential energy (hydraulic head) drive groundwater flow. Therefore, preparing a groundwater contour map will give better insights into the local groundwater flow regimes. It was possible to convert water depths (m bgl), when wellhead elevations are measured using a *Trimble* Global Positioning System (GPS), to water level elevations for use in construction of water table contour maps. The lines of equal hydraulic head are called equipotential lines that indicate the water flow direction across a catchment as flow occurs perpendicularly to those lines, called flow lines (Bartley and Johnston, 2006). Generally, groundwater flow follows topography; in reality, the situation can be more

complicated however. Groundwater flow not only occurs near the water table, but does penetrate deep into the aquifer (Freeze and Cherry, 1979). The hydraulic gradient was estimated as the hydraulic head difference over distance:

$$i = \frac{\Delta h}{\Delta l} \qquad (Eqn.5.8)$$

where i is hydraulic gradient, Δh is the head difference between two adjacent wells and Δl is the difference in distance between the two wells.

According to Darcy's Law (1956), groundwater hydraulic conductivity is also a function of hydraulic gradient (Eqn. 5.9).

$$Q = (K_{sat} A \frac{\Delta h}{\Delta l}) \qquad (Eqn.5.9)$$

Q is the groundwater flux ($m^3\ d^{-1}$), K_{sat} is saturated hydraulic conductivity ($m\ d^{-1}$), Δh/Δl is the natural hydraulic gradient along the horizontal flow direction distance between a given well pair, and A is the saturated unit area thickness (m^2). Saturated area for each well was estimated by multiplying the saturated depth by a width of 1m to enable comparisons among well (Lowrance et al., 1984). Hölting and Coldewey (2005) stated that high hydraulic gradient coincides with low hydraulic conductivity. Generally, the closer are the positions of wells on the isolines, the higher the hydraulic gradients, and the smaller the hydraulic conductivity. Therefore, groundwater contour maps can indicate the spatial distributions of hydraulic conductivity across catchment. However, the slope of the potentiometric surface only provides an approximate direction for the driving forces of groundwater flow, whereas the fracture network pattern controls the actual flow path (Nativ et al., 2003). The groundwater isolines maps for each site were drawn using SURFER (version 8, Golden Software), a contouring and surface mapping programme.

5.4.2 Results and discussion

The water elevation contour map of JC revealed that groundwater flows from JC1A, JC1B and JC1C towards JC32, JC33 and JC34 across the catchment (Figure 5.13). The GWT in the wells JC1A, JC1B and JC1C are located at approximately 58 m AOD and GWT in the wells JC32, JC33 and JC34 are located very close to the stream (29.56 m AOD) as of Jan, 2010. Groundwater sampling along its flow paths thus can help understanding the nitrate retention by denitrification across the catchment (grazed grassland) while passing through

the saturated soils and sediments. The hydraulic gradient at JC site ranged from 0.002 to 0.032, indicating a wide range of hydraulic conductivity across the catchment, generally higher on the top slope of the field to lower on the down slope (Appendix 5). Because, generally the narrower the isolines, the higher the hydraulic gradients and lower hydraulic conductivity.

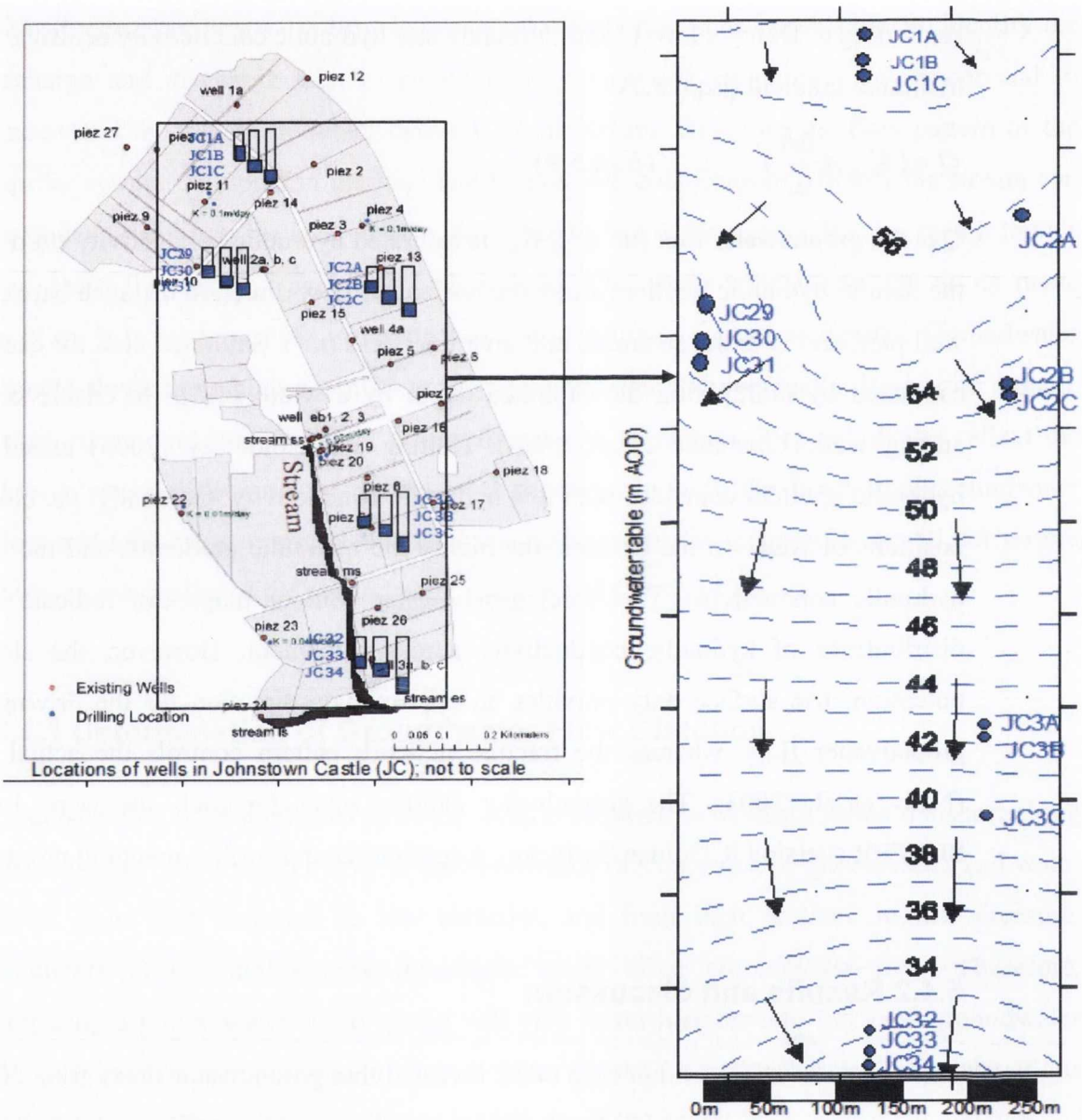
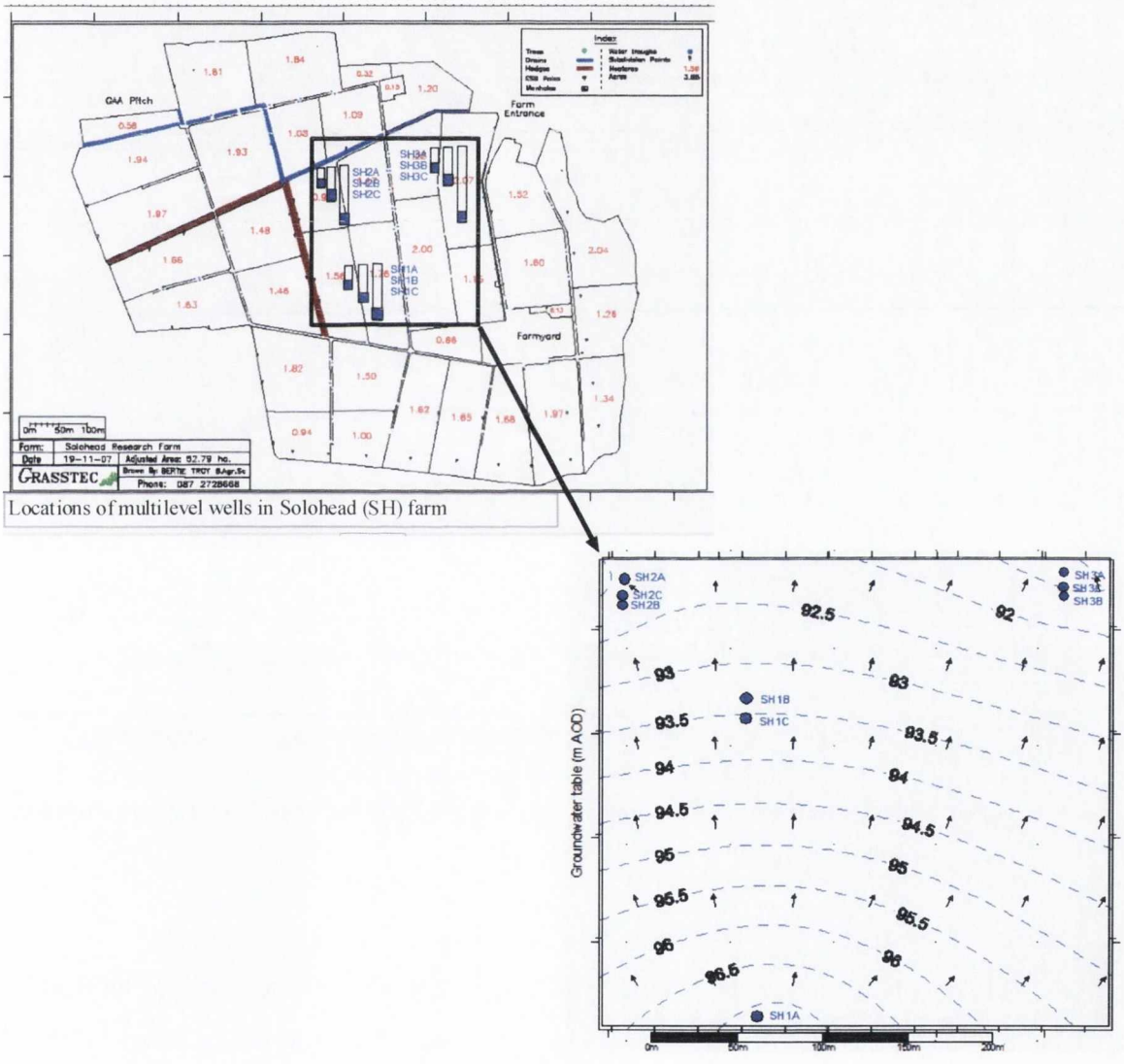


Figure 5.13 Groundwater contour and locations of wells at Johnstown castle dairy farm; well IDs are shown on an existing map with other well (as of January 2010)

At the SH site, GWT in the top hill well (SH1A) is located approximately at 98 m AOD. The two wells (SH1B and SH1C) located at the same place of SH1A but water table/ potentiometric levels of these two wells are located at 93-94 m AOD. The down most GWT was recorded in well SH3A, SH3B and SH3C (approximately 92 m AOD). Therefore, the groundwater contour indicates the flow of groundwater from SH1A, SH1B and SH1C to SH2A, SH2B, SH2C, SH3A, SH3B and SH3C (Figure 5.14). The length of the field is approximately 300 m (grazed grass plus clover). The isolines are approximately similar in depths (except SH2B and SH2C) which indicated that hydraulic gradient and hydraulic conductivity across the field were approximately similar.



Locations of multilevel wells in Solohead (SH) farm

Figure 5.14Groundwater contour and locations of wells at Solohead dairy farm; well IDs are shown on an existing map with other well (as of January 2010)

At OP site, groundwater flows from wells OP1A, OP1B, and OP1C where GWT is located at approximately 54 m AOD towards the wells OP2A, OP2B and OP2C where GWT is at 52 m AOD (Figure 5.15). The distance between these two groups of wells are approximately 474 m. The field is under arable land with spring barley and mustard rotations.

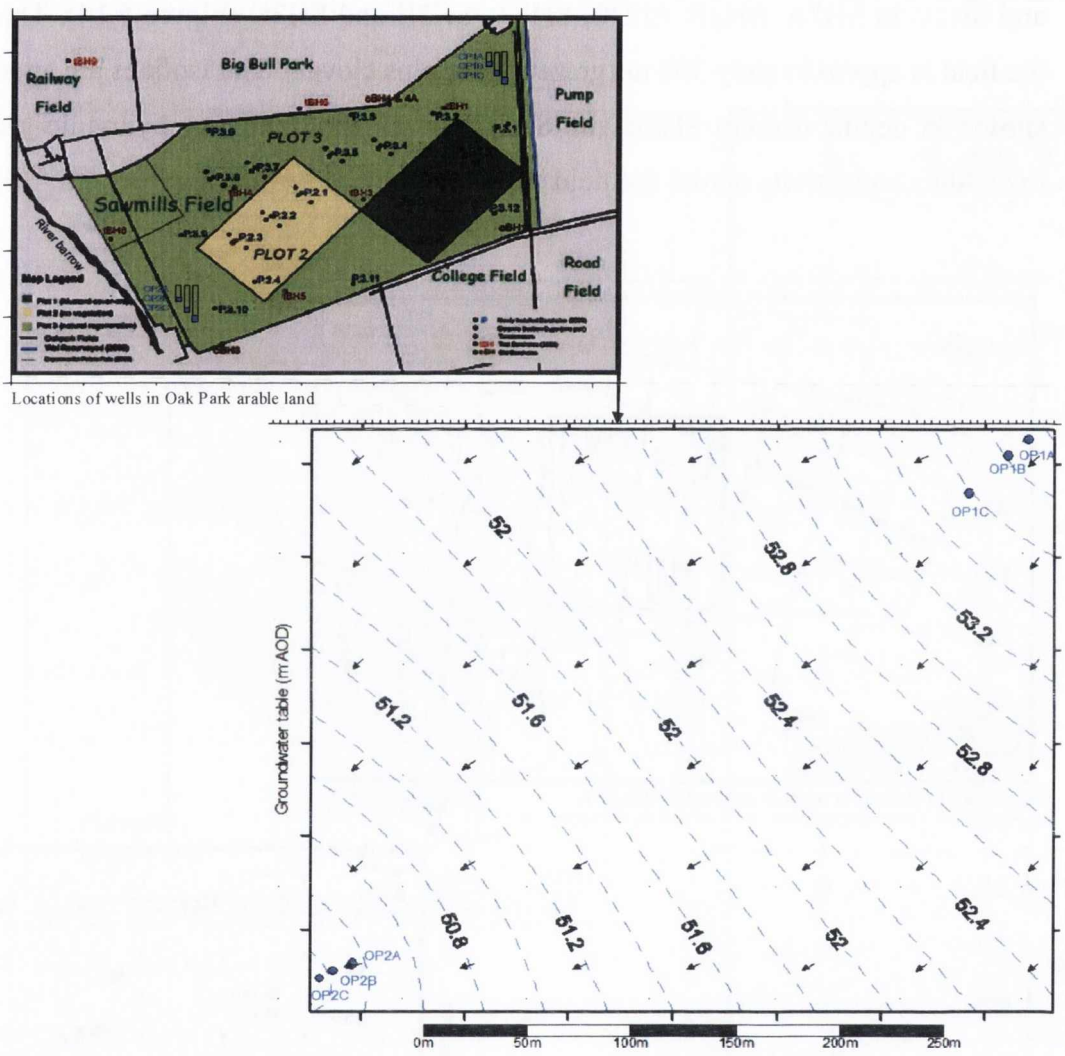


Figure 5.15Groundwater contour and locations of wells at Oak Park; well IDs are shown on an exiting map with other well (as of January 2010)

At DG site, groundwater shows multidirectional flow where GWT at DG104 is located at the top most level of the field (31 m AOD) and DG106 (19.5 m AOD) at the down most level (Figure 5.16). All the wells across the catchment are located in intensively managed grazed grassland which receives substantial quantity of surplus N which poses risk of groundwater contamination with dissolved organic and inorganic N. However,

groundwater sampling along these flow paths can help understanding the fate of applied surplus N and the risk of delivery to the river. The distributions of isolines (lines of similar potential) across the field are indicating a very heterogeneous system with varying hydraulic gradients and hydraulic conductivities. The wells, DG104 and DG105 showed comparatively narrower isolines than other wells indicating that these two wells have comparatively higher hydraulic gradients (Appendix 5) and lower hydraulic conductivities.

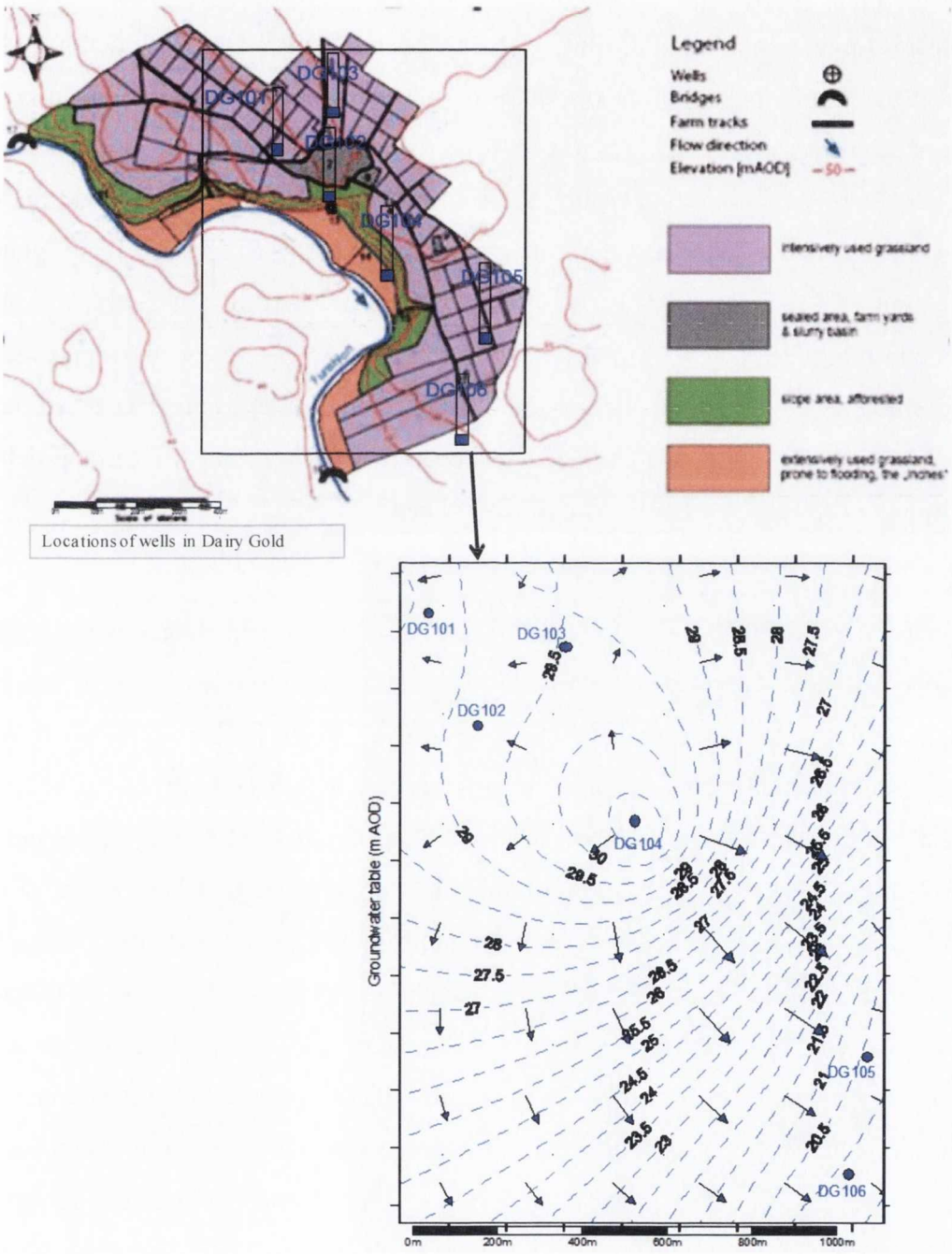


Figure 5.16 Groundwater contour and locations of wells at Dairy Gold dairy farm; well IDs are shown on an existing map with other well (as of Jan 2010)

5.4.3 Conclusions

The study was conducted in two hydrologically contrasting years where 2010 was dryer (coupled with higher SMD and lower ER) than 2009. The percentage of rainfall that became ER was also lower in 2010 than in 2009. The GWT showed clear response to the ER showing shallowest during October to December and deepest during July to September. The four sites showed very contrasting hydrologic regimes with lowest depth of unsaturated zone at SH to highest at DG. The shallowest GWT depth was consistent with the lowest permeability of aquifer. In 2010, despite a low ER, mean GWT did not drop significantly (except at DG) may be due to contribution of groundwater upwardly from the deeper aquifer suggesting the confined nature of bedrock zone. In the wetter year (2009) mean GWT did not even increased significantly, suggesting that higher hydraulic loading can increase the rate of groundwater flow towards the surface waters or can maintain higher downward pressure to prevent the upward flow of groundwater from the confined layer. Therefore, N management in agricultural lands can be carried out following the rainfall, ER, SMD and the GWT response to reduce the risk of nutrient delivery to the groundwater and eventually to the surface waters.

Hydraulic conductivity is very closely connected to the hydrogeological and biogeochemical environments in the field and therefore is of great implication for agricultural water management. High hydraulic conductivity clearly shows its implications on risk of contaminants delivery to groundwater and eventually to surface waters. The results from the present research imply that any liquid fertilizer e.g., farm gate wash out, soiled water, slurry application to the high permeable grassland dairy farming (DG) especially in winter will pose serious risk of groundwater contamination with nitrate due to the free drainage nature of soils/sediments. It is mainly because NO_3^- produced by nitrification from the applied fertilizers or manures will have less residence time to be biogeochemically processed, so as to why it will be delivered to groundwater and then to surface water before it is denitrified. Moreover, high hydraulic conductivity can cause high DO, equilibrated with the percolating water which eventually enriches groundwater with DO (Appendix 9). This hypothesis is supported by the highest existence of NO_3^- and DON concentrations at DG site (Appendix 11). Conversely, low permeability offered high residence time for nitrate, produced by nitrification in unsaturated zone, to under go further

transformations to NH_4^+ and N_2 while it is passing through and from the landscape towards the receptors. At two other grassland dairy farming systems (JC and SH) in this study, very low range of nitrate and DON concentrations coupled with substantial quantity of excess N_2 (Appendix 11) can be an example of high biogeochemical transformations of nitrate, even though their management systems are quite similar.

5.5 Linking Hydrogeochemistry to the Abundance of NO_3^- in Groundwater

5.5.1 Background

Nitrate concentration in groundwater, however, does not necessarily remain constant and is a function of several physical and biogeochemical processes e.g., dispersion, denitrification, microbial assimilation, immobilization, DNRA, anammox etc. Of the biogeochemical processes, denitrification is the principal process which converts the reactive N to N_2 (Rivett et al., 2008). The organisms that contribute to denitrification are ubiquitous in surface water, soil and groundwater (Beauchamp et al., 1989); they are found at great depths in aquifers e.g., nearly 300 m below ground (Francis et al., 1989). Therefore, NO_3^- reduction should mainly be controlled by hydrological and geochemical factors. Denitrifiers are facultative anaerobic heterotrophs (obtain C and energy from oxidation of organic compounds e.g. organic C) and autotrophs (obtain energy from oxidation of inorganic compounds e.g. reduced S or Fe). In the denitrification process organic C and/or reduced Fe or Mn can act as electron source and NO_3^- as electron acceptor. Multiple electron donors can contribute to NO_3^- reduction by denitrification (Rivett et al., 2008; Böhlke et al., 2002). Therefore, investigation of the abundances of electron donors in subsurface environments can give insights into the abundances of NO_3^- in groundwater and its subsequent delivery to surface waters. Denitrification is principally an anaerobic process which starts at an oxygen level 4 mg L^{-1} (Böhlke and Denver, 1995); 2-3 mg L^{-1} (Tang and Sakura, 2005). However, the oxygen level ranges require more consensuses (Buss et al., 2005). The redox chemistry is an important phenomenon that can be used as an indication of environments favourable for denitrification (Buss et al., 2005). Local hydrology and hydrogeochemistry e.g., GWT fluctuations, water flow and temperature; and pH and common ions may control the concentrations of NO_3^- in groundwater. A process-based understanding of the factors controlling the abundances of

nitrate and of their distributions over space and time is crucial for quantifying the effects of human activity on the N cycle and for managing and mitigating the severe environmental consequences associated with N pollution (Boyer et al., 2006). In the work now presented, groundwater hydrologic and geochemical variables at 4 agricultural catchments in Ireland were investigated in the context with the potential implications for the abundances of nitrate in three distinct subsurface zones (i.e., vertical profile).

5.5.2 Hydrogeochemical Characterization

5.5.2.1 Frequency of groundwater sampling

Groundwater sampling was carried out monthly between Feb, 2009 and Jan, 2011 using a bladder pump (Geotech Environmental Equipment, Inc., USA) following USEPA Region I Low Stress Purging and Sampling Procedures (USEPA, 1996) for analysing dissolved gases and hydrochemistry. Groundwater pH, temperature, turbidity, DO, electrical conductivity and Eh were measured on-site using an In Situ Multiparameter Probe (In Situ Inc. USA). Triplicate samples were collected through Teflon outlet tubing (ID 0.6 cm) at a slow rate of 100 ml min^{-1} to avoid ebullition of dissolved gases during sampling. To analyse dissolved CO_2 and CH_4, water samples were collected in 160 ml serum bottles by slowly overflowing of approximately 150 ml water and then immediately sealing with butyl rubber septa and aluminium crimp caps (WHEATON, USA), were kept (stored under water) in cool box and stored at 4°C and analysed within one week. Due to low flow pumping, no visible air bubbles were observed in water samples. However, a drop in the hydrostatic pressure of groundwater during collection through small-diameter pipe can cause spontaneous ebullition of gas bubbles (degassing). Moreover, when atmospheric concentrations of these gases are abundant relative to the dissolved gas concentrations, contamination during sample collection is a concern. Preliminarily groundwater samples were collected from 6 wells into pre-evacuated and non-evacuated serum bottles with 3 replications and statistically tested if there was any significant difference between the two methods. The preliminary experimentation on collecting samples in pre-evacuated and non-evacuated serum bottles showed no significant differences for dissolved gases.

5.5.2.2 Hydrogeochemical Analyses

Groundwater non-metallic ions e. g. total oxidised N, nitrite, NH_4^+, Cl^-, and P; reduced metals e.g. Fe^{2+}, Mn^{2+} and S^{2-} were analyzed with an Aquakem 600 Discrete Analyser (Aquakem 600A, Vantaa, Finland). Metallic ions were analysed on an axial inductively coupled plasma spectrophotometer (ICP), the model used being a Varian Vista-MPX CCD-Simultaneous ICP-OES. Analysis followed instrument manufacturer's procedures and all calibration standards were made from certified stock standard solutions. Table 1 shows the manufacturer's instrument detection limits for axial ICP.

Sulphate, DOC and Total nitrogen (TN)

Groundwater SO_4^{2-} concentration was measured with a turbimetric method (Askew and Smith, 2005a). DOC was analysed using a Total Organic Carbon Analyser (TOC-V cph/cpn; Shimadzu Corporation, Kyoto, Japan) with ASI-V autosampler. Calibration curves, using standard concentrations 0, 3.33, 6.67 and 10 mg L^{-1}, were obtained by automatic serial dilution of a 1000 mg L^{-1} organic carbon certified stock standard with ultra pure water (Baird, 2005). TN was analysed by catalytic combustion method (Goldman and Clifford, 2008). When a sample is introduced into the combustion chamber (720°C) total nitrogen compounds in the sample decompose to nitrogen monoxide (NO). Nitrogen gas is not affected by this process. The NO content is then analysed by chemiluminescence. Another gas phase reaction is the basis of nitric oxide detection in commercial analytic instruments applied to environmental air quality testing. Ozone is combined with nitric oxide to form nitrogen dioxide in an activated state. The activated NO_2^- luminesces broadband visible to infrared light as it reverts to a lower energy state. A photomultiplier and associated electronics counts the photons which are proportional to the amount of NO present. Total phosphate was analysed using a persulfate method (Eaton et al., 2005).

5.5.2.3 Analysis of dissolved CO_2 and CH_4

To determine the dissolved CO_2 and CH_4 concentrations, samples were degassed (Lemon, 1981; Davidson and Firestone, 1998) using high purity He (BOC, Linde Group, Germany) (*He*: water 1:3; v/v). The headspace volume was augmented to 40 ml by an additional injection of 40 ml of *He* and simultaneous replacement of 40 ml water through the rubber

septum of sealed serum bottle using plastic syringe. The needle was connected to Cu tube (which was connected to the gas cylinder) with a 2-way valve. The samples were shaked with a mechanical shaker for 5 min at 400 rpm and left standing for 30 min. After equilibration, headspace gas sample was extracted into 12 ml exetainer (Labco, Wycombe, UK) with an additional injection of 12 ml *He* using PVC syringe. The CO_2 and CH_4 was analysed in auto sampler gas chromatograph (CP-3800, Varian, Inc. USA) equipped with TCD and FID, respectively, using Ar as a carrier gas. Calculation of CO_2 and CH_4 was carried out using Henry's Law with the solubility co-efficients of the gases at ambient groundwater temperature (Appendix 6). In addition, an experiment was carried out to compare different methods used to extract dissolved gases in groundwater with a view to evaluate whether there is any possibility to underestimate the gas extraction and explained in Appendix 7).

5.5.2.4 Calculation of dissolved CO_2 and CH_4

The USEPA (RKSOP, 2004) describes a precise method for degassing groundwater to analyse dissolved gases. According to Henry's law, the equilibrium value of the mole fraction of gas dissolved in a liquid is directly proportional to the partial pressure of the gas above the liquid surface. This implies that when a headspace is created above a water sample, gases which are in the water will equilibrate between the headspace and the aqueous phase. The details step by step procedures were explained in Appendix 6.

5.5.2.5 Statistical analysis for hydrogeochemical parameters

Analysis of data was performed using the Mixed Procedure (SAS, 2009). As most of the variables showed an approximately lognormal distribution, log transformations were used with appropriate re-scaling so that residual checks indicated that the assumptions of the analyses were not violated. Pre-specified hypotheses of influential variables were tested by regression modelling for NO_3^--N. Sequential addition of the variables to the model was performed where the size of the F statistic gives an indication of their relative contribution to the full model. Structural factors like depth and sampling dates were tested. Covariance models were included to account for correlations in the data (e.g. across sampling date). For each hydrological and geochemical parameter effects of location and depth were

examined along with their interactions. In case significant differences were found, Tukey Kramer HSD multiple comparison test were used to distinguish differences between individual site and depth.

5.5.3 Hydrogeochemical properties across study sites

5.5.3.1 Temperature

Groundwater temperature was approximately similar across sites and depths with low spatial and temporal variability. It ranged from 11.1-11.3, 10.9-11.0, 10.4-10.6 and 10.0 °C respectively at JC, SH, OP and DG with corresponding mean values of 11.2, 11.0 and 10.5 and 10.0°C over the two years (2009-2010). Monthly temperature fluctuations were observed in subsoil and at bedrock-interface, being higher in subsoil than at bedrock-interface, but in bedrock it was quite stable in all sites (Figure 5.17). Temperature changes were also recorded in every 30 min over the two years study period and presented in Appendix 8. Interestingly, the timing of the peaks and troughs in the temperature record over time (highest and lowest valued recorded) was different in subsoil and at bedrock-interface. In subsoil, the highest temperature at each site in each year was recorded during July to August and lowest during February to March. At the bedrock-interface the highest temperature (12-13°C) was recorded during September to October and lowest during February to March (5.5-8.0° C).

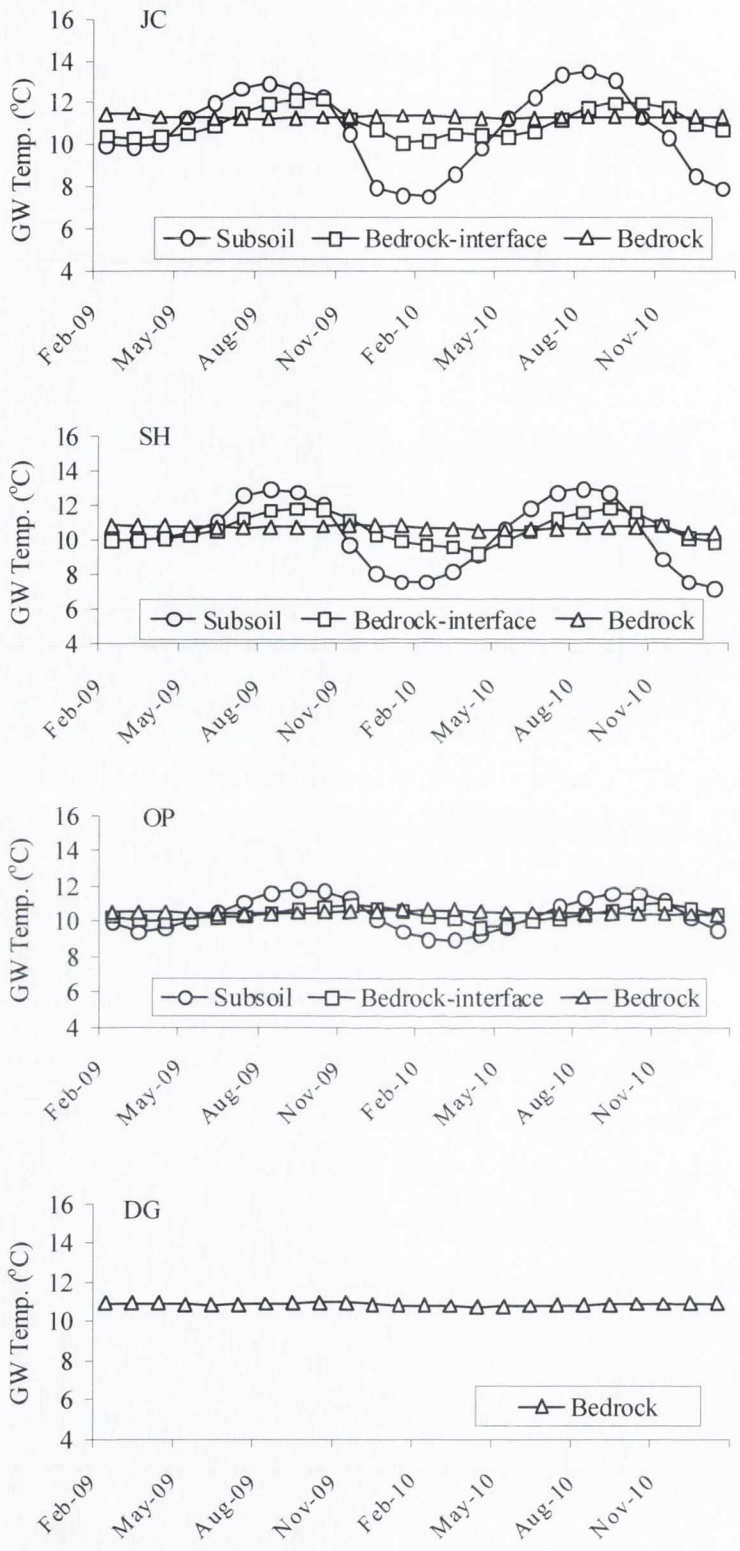


Figure 5.17Groundwater temperature in three different depths; subsoil (5 m bgl), bedrock-interface (12 m bgl) and bedrock (22-30 m bgl) at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park, and in only bedrock at (DG) Dairy Gold

5.5.3.2 pH

Groundwater pH was neutral to alkaline (mean pH 6.8 - 7.9) across sites (Figure 5.18) and was significantly different between sites ($p<0.001$). Considering the differences among depths of groundwater, pH was similar in all depths except OP where it was lower in subsoil and bedrock than bedrock-interface ($p<0.001$). The variability in pH over time was moderate with the mean coefficients of variation across depths ranging from 4-7, 4-5, 5-23 and 4%, respectively, at JC, SH, OP and DG sites. Groundwater pH in wells at each site is presented in Appendix 9.

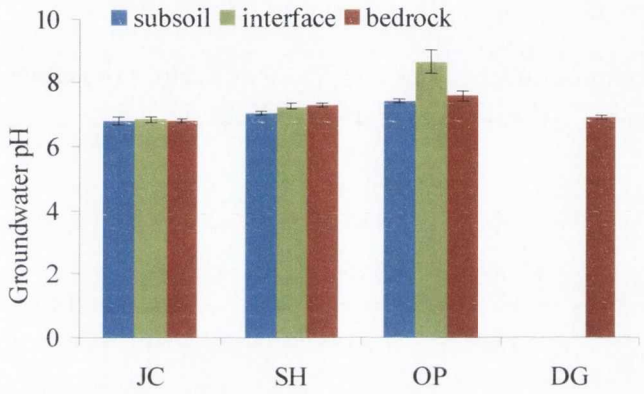


Figure 5.18 Groundwater pH in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n = 24)

5.5.3.3 Dissolved Organic C (DOC)

Dissolved organic C (DOC) concentration in groundwater did not differ between sites and depths ($p>0.05$). Mean DOC concentrations were 2.3-4.0, 1.1-1.6, 0.6-1.1 and 0.9 mg L^{-1}, respectively, at JC, SH, OP and DG, irrespective of depth (Figure 5.19). Despite a numerically higher mean value at JC, it was similar across sites because it showed high spatial variability within the JC site study area. The DOC in 3 wells at JC (i.e. JC2A, JC2B and JC2C) was unusually higher (8.81-15.90 mg L^{-1}) than that observed in all other wells (Appendix 9). Land around these wells has been irrigated with dirty water (farm yard washout) for approximately ten years (Section 7.1.6.3). The DOC showed high temporal variability with highest concentrations during December to January and lowest during August to September with the coefficients of variation among depths 147-159, 75-91, 54-

99 and 56%, respectively, at JC, SH, OP and DG. Groundwater DOC between wells at each site was presented in Appendix 9.

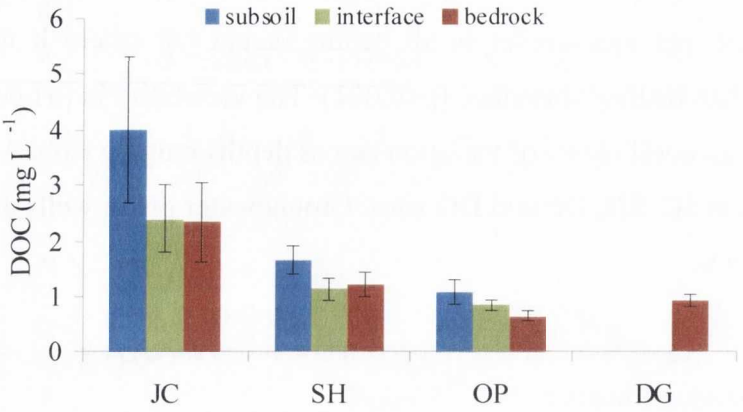


Figure 5.19 Groundwater DOC in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n = 24)

5.5.3.4 Dissolved CO_2

Dissolved CO_2, an important greenhouse gas, showed significant variations between agricultural land uses i.e., sites located in grassland and arable land. Significantly higher CO_2 concentrations were measured ($p<0.001$) in grassland than in arable land. Moreover, CO_2 concentrations decreased significantly ($p<0.001$) with depths (Figure 5.20). Mean CO_2 concentrations were 35.5, 27.6, 11.6, and 33.1 mg C L^{-1}, respectively, at JC, SH, OP and DG, and showed large spatial variability at each site (Appendix 9). In general, higher CO_2 concentrations in groundwater were measured during July to September than those measured at other times of the year. Similar to the other hydrochemical properties, CO_2 production showed moderate temporal variations at each site with mean CV values of 44, 85, 74 and 34%, respectively, for JC, SH, OP and DG.

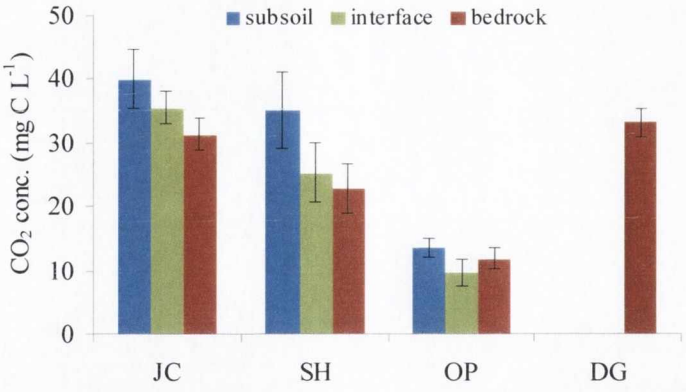


Figure 5.20Groundwater CO_2 in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n = 24)

5.5.3.5 Dissolved CH_4

Dissolved CH_4 production was quite intermittent across sites and depths. CH_4 production was observed at all sites, but its frequency of occurrence and the magnitude of concentrations observed were quite different. CH_4 production was higher than the detection limit in 35 and 60% of the wells at JC and SH study sites, respectively but in other wells at these sites and at other two sites (OP and DG) it was close to the detection level showing the mean values of 246.5, 29.9, 5.0, and 1.3 µg C L^{-1}, respectively, at JC, SH, OP and DG (Figure 5.21). Subsoil showed lower CH_4 production than interface and bedrock (Figure 5.18). Temporal changes in CH_4 concentrations were even higher than the CO_2 production showing the highest concentration during August to October and lowest during December to February. The mean CV values were 360, 300, 279 and 78%, respectively, at the JC, SH, OP and DG sites. The CH_4 concentrations were highly variable between wells at each site (Appendix 9).

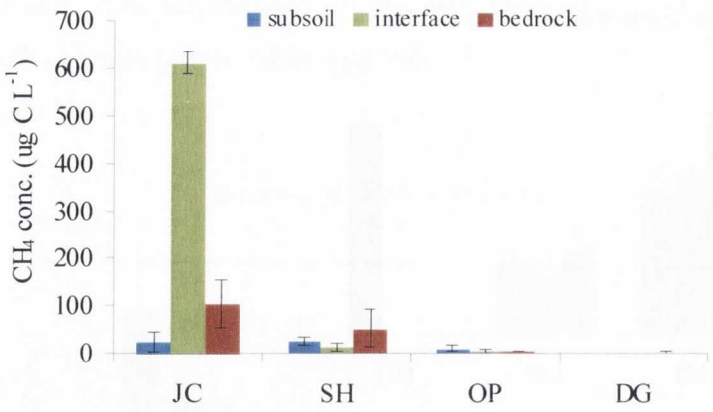


Figure 5.21 Groundwater CH_4 concentrations in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n=24)

5.5.3.6 Available Sulphate (SO_4^{2-}) and Reduced Sulphite (S^{2-})

In terms of the geographical location and land use, SO_4^{2-} concentrations in groundwater were similar ($p>0.05$) at all sites except OP where it was significantly higher ($p<0.05$) than those values recorded at other sites. However, in the vertical dimension, SO_4^{2-} concentrations at JC and SH were significantly higher ($p<0.05$) at interface and bedrock than in subsoil. Mean SO_4^{2-} concentrations over the 2- year monitoring period were 18, 15, 26 and 20 mg L^{-1}, respectively, at JC, SH, OP and DG, irrespective of the depth (Figure 5.22). The SO_4^{2-} concentration across sites were slightly higher than the natural background level (NBL) 18 mg L^{-1} except at the SH site, but were lower than the environmental quality standard (EQS) of 187.5 mg L^{-1}. It had moderate temporal variability with consistently higher values during July to September and lower during December to February showing mean coefficient of variations of 40-57, 32-76, 13-23 and 33%, respectively at JC, SH, OP and DG. The CV values were comparatively higher at JC and SH sites because there are some wells that have comparatively higher SO_4^{2-} concentrations e.g. JC1A, JC29, JC30, JC31, JC2A, JC2B, JC2C, JC3A, JC3B; SH1A, SH2B, SH2C, SH3A, SH3B and SH3C (Appendix 9).

Reduced S (S^{2-}) concentrations were similar across sites and depths ($p<0.05$) with the mean values of 0.24, 0.19, 0.20 and 0.14 mg L^{-1} (Figure 5.22). Reduced sulphur concentrations generally decreased with increasing depth in the groundwater profile except at the OP site

where it was higher at interface than in subsoil and bedrcok. There was a significant negative relationships between sulphide and sulphate concentrations (r=-0.35; p>0.05). The mean coefficients of variation over the sampling period were 116, 126, 118 and 125%, respectively at JC, SH, OP and DG.

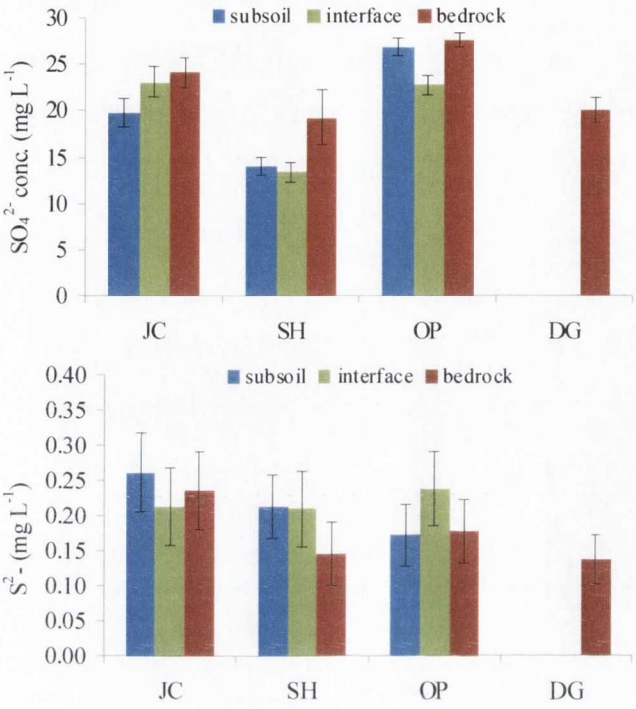


Figure 5.22Groundwater SO_4^{2-} (top) and S^{2-} (bottom) in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n=24)

5.5.4 Groundwater redox chemistry

5.5.4.1 Dissolved Oxygen (DO)

The DO concentrations showed very contrasting results across depths and sites (Figure 5.23). Mean DO was similar at JC and SH but were significantly higher at DG (8.7 mg L^{-1}) and OP (7.1 mg L^{-1}) than at JC (1.7 mg L^{-1}) and SH (1.4 mg L^{-1}). However, in bedrock, it was significantly higher at DG (8.7 mg L^{-1}) than at JC (1.5 mg L^{-1}), SH (1.3 mg L^{-1}) and OP (4.8 mg L^{-1}) ($p<0.001$). Considering the differences in DO among depths at an individual site, significantly higher DO concentrations were observed in subsoil than at the interface and bedrock at JC, whereas it was similar among depths at OP and SH. Very

interestingly, DO at interface was similar across sites. A high temporal variability in DO concentrations was observed over the sampling periods with being higher during November to January and lower during July to September, irrespective of sites and depths. The mean coefficients of variations over the sampling period ranged among depths from 74-127, 62-113, 49-62 and 28%, respectively at JC, SH, OP and DG sites. A typical example of the temporal changes in DO concentrations at JC site is shown in Figure 5.24 as similar pattern of changes was observed at all sites. The DO showed a significant and negative linear correlation with the depth of unsaturated zone (revealed by the ratio of depth bwt to depth bgl) and positive with K_{sat} (Figure 5.25).

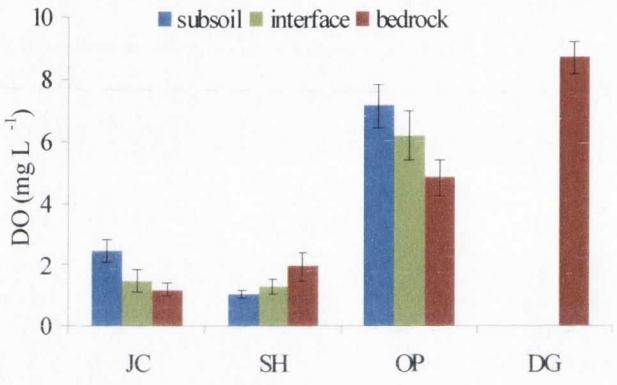


Figure 5.23Groundwater DO in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n = 24)

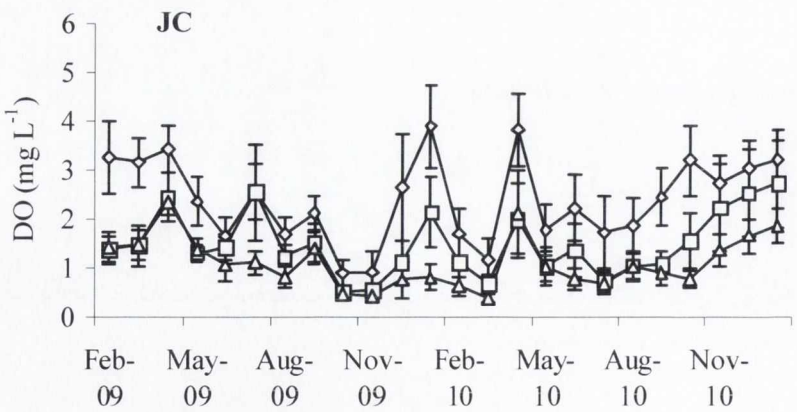


Figure 5.24Temporal changes in DO concentrations at Johnstown Castle in three different depths of groundwater (mean ± SE; n = 5)

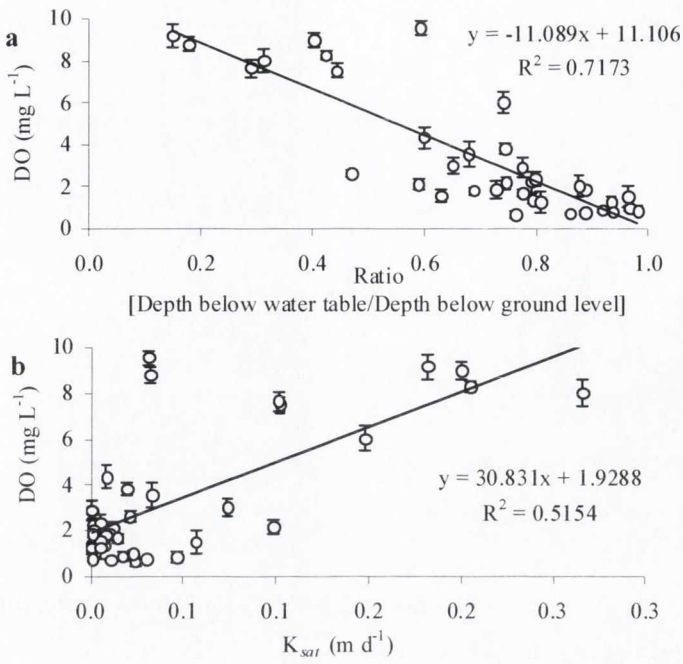


Figure 5.25Plots; groundwater DO vs. (a) depth below GWT/depth bgl (n=36), and (b) K_{sat} (mean ± SE; n=36)

5.5.4.2 Redox potential (Eh)

Mean Eh distributions across sites and depths showed similar pattern to DO concentrations. Over the two years (2009-2010), mean Eh was significantly different between sites ($p<0.001$) and depths ($p<0.01$). Irrespective of depths of groundwater, it ranged from 51-107, 42-92, 120-160 and 176 mV, respectively, at JC, SH, OP and DG (Figure 5.26). There were some wells at JC (JC2B, JC2C, JC33, and JC34) and SH (SH2A, SH2B, SH3A, and SH3B) where Eh ranged from -1 to -72 mV, suggesting comparatively anaerobic environment in groundwater (Appendix 5). Mean coefficients of variation at each site were medium to high, which ranged between depth from 68-217, 83-250, 42-76 and 40%, respectively, at JC, SH, OP and DG sites. The Eh showed significant positive correlation with permeability of aquifer and negative correlation with depth of unsaturated zones (normalized with ratio of depth bwt to depth bgl) (Figure 5.27).

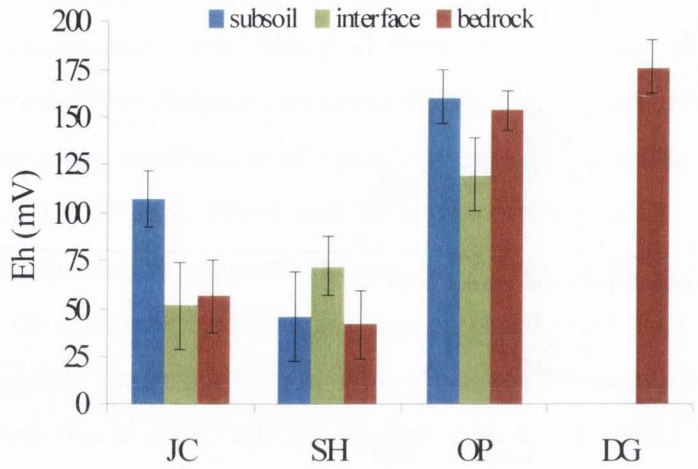


Figure 5.26Groundwater Eh in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n = 24)

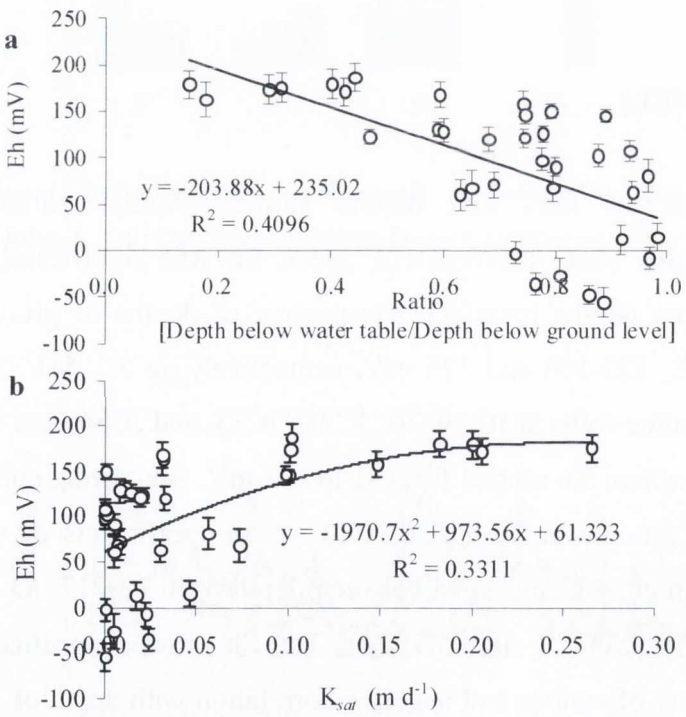


Figure 5.27Plots; groundwater Eh vs. (a) depth below GWT/depth bgl (n = 36), and (b) K_{sat} (mean ± SE; n = 36)

5.5.4.3 Total and Reduced Iron (Fe and Fe^{2+})

Total Fe concentrations were different at each site with the highest mean values over the study period at JC (480 μg L^{-1}) and lowest at DG (45 μg L^{-1}). The JC and SH sites had significantly higher total Fe concentrations than the OP and DG sites (Figure 5.28). No significant differences were observed between JC and SH and between OP and DG sites. However, it showed large spatial variability (Appendix 10) at each site. Temporal changes during the two years were even higher than the spatial variability with the coefficients of variation of 278, 215, 219 and 308% at JC, SH, OP and DG, respectively. Reduced iron (Fe^{2+}) concentrations were significantly higher at JC and SH than OP and DG ($p<0.001$) with the mean values 30.7, 26.0, 1.2 and 10.4 μg L^{-1}, respectively, at JC, SH, OP and DG. It showed similar concentrations across depths ($p>0.05$). High temporal variability of Fe^{2+} was observed at all sites with coefficients of variation 218, 111, 98 and 201%, respectively at JC, SH, OP and DG. Total and reduced Fe data across sites and depths were log-normally distributed.

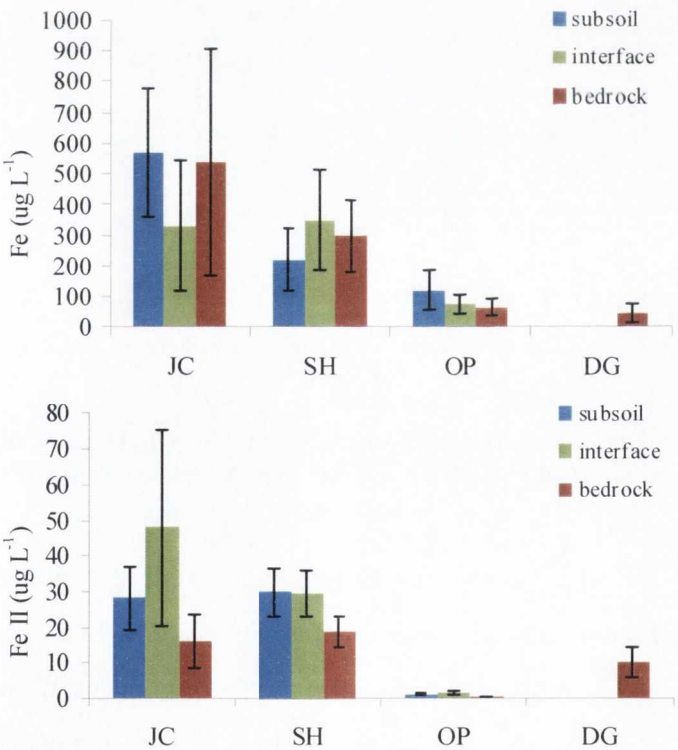


Figure 5.28Groundwater total Fe (top) and reduced Fe (bottom) in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n=24)

5.5.4.4 Total and Reduced Manganese (Mn and Mn^{2+})

The distributions of total and reduced Mn concentrations showed similar patterns to total and reduced Fe concentrations. Mean total Mn concentration was 301, 130, 9 and 5 μg L^{-1}, respectively at JC, SH, OP and DG (Figure 5.29). Mn^{2+} concentration showed similar phenomenon to Fe^{2+} with significant differences between sites ($p<0.05$) but similar concentrations between depths. Mean Mn^{2+} concentration was 171, 46, 5 and 3 μg L^{-1}, respectively at JC, SH, OP and DG with coefficients of variation over time 167, 106, 198 and 178%.

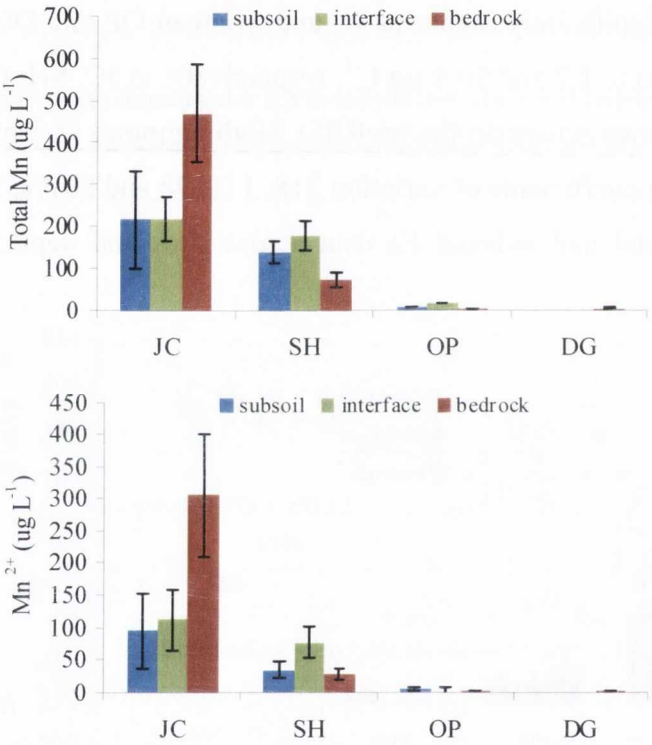


Figure 5.29 Groundwater total Mn (top) and reduced Mn (bottom) in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n=24)

The Eh in groundwater showed good correlations with reduced metals and DO concentrations (Figure 5.30), being positive with DO but negative with Fe^{2+} and Mn^{2+} and quadratic with S^{2-}. It was also inversely correlated with the DOC concentrations ($r=-0.334$; $p<0.023$). Very interestingly, Fe^{2+} and Mn^{2+} started to increase in groundwater while Eh drops below 150 mV and reached maximum levels while Eh drops below 100 mV. The S^{2-} concentrations decreased with the increase in Fe^{2+} and Mn^{2+} concentrations in some of the wells at JC and SH sites.

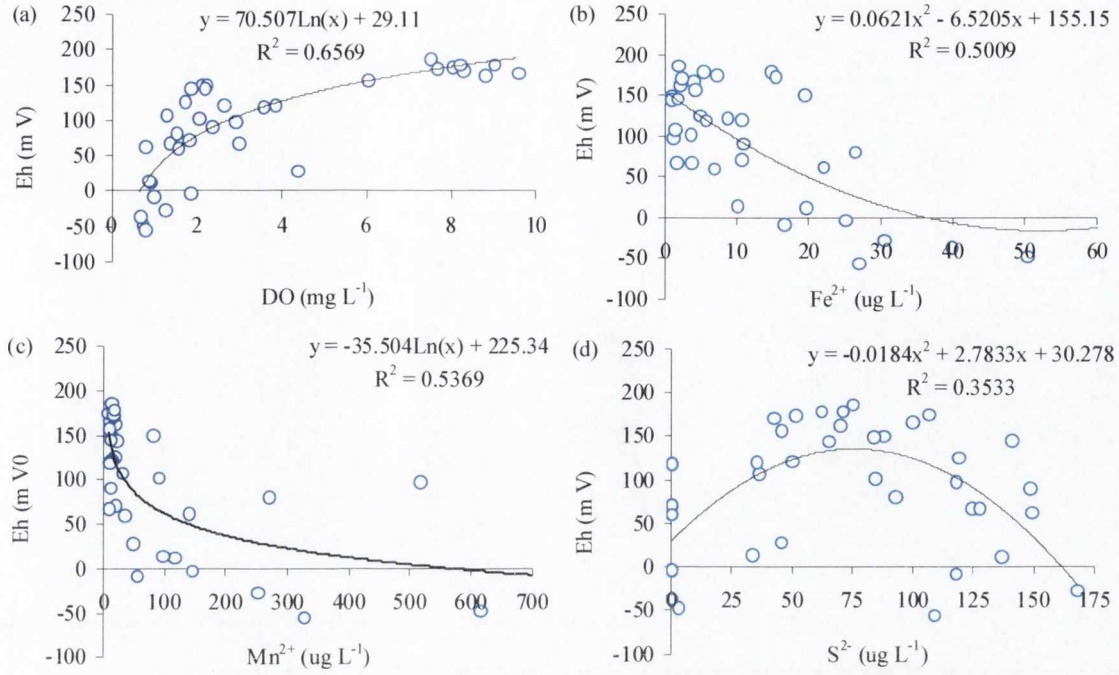


Figure 5.30Plots, Eh vs. (a) DO, (b) Fe^{2+}, (c) Mn^{2+} and (d) S^{2-} (n = 36)

5.5.5 Groundwater N dynamics

5.5.5.1 Nitrate (NO_3^--N)

Groundwater mean NO_3^--N distributions in different depths across sites are shown in Figure 5.31. Mean NO_3^--N concentrations over the two years (2009-2010) were 3.7, 0.7, 11.0, and 14.6 mg N L^{-1}, respectively, at JC, SH, OP and DG which were significantly different between sites ($p<0.001$) and depths ($p<0.01$). Mean NO_3^--N concentrations at OP and DG sites were higher than the EQS, 8.47 mg N L^{-1}. Moderate temporal variability of NO_3^--N concentrations were observed across sites and depths (Appendix 11) with higher NO_3^--N concentrations during December to February and lower during August to October, in general. Mean coefficients of variation over time were 62-86, 103-149, 30-336 and 42% respectively at JC, SH, OP and DG sites. NO_3^--N concentrations between wells at each site showed high spatial variability ranging from 0.3 to 8.2, 0.2 to 4.5, 4.5 to 15.0 and 7.5 to 26.0 mg N L^{-1}, respectively at JC, SH, OP and DG (Appendix 11). Very low concentrations of nitrate (close to the detection limit of 0.02 mg N L^{-1}) were recorded at some of the wells at the JC site, at the bedrock-interface and in the wells targeting the

bedrock (JC2B, JC2C, JC33 and JC34). Similarly, low concentrations of nitrate were recorded at most of the wells at the SH site in all depths.

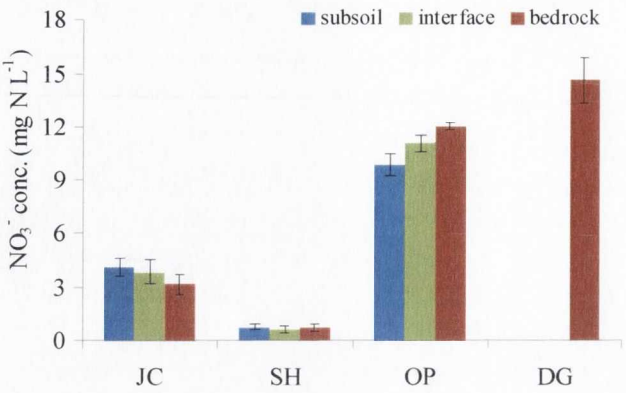


Figure 5.31Groundwater NO_3^--N in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n = 24)

5.5.5.2 Nitrite (NO_2^-) and ammonium (NH_4^+)

Concentrations of NO_2^- at JC and OP were very low (almost trace) with 0.01-0.06 and 0.02-0.38 mg N L^{-1} but at SH and DG most of the sampling times it was close to detection limit. OP site showed significantly higher NO_2^- concentrations than JC ($p<0.001$) (Figure 5.32). On a few occasions, the NO_2^- concentrations at the JC site were higher than the EQS. However, at the OP site NO_2^- concentrations exceeded the EQS on most of the sampling occasions.

Ammonium concentration was detected at all sites and depths of the investigated profile. However, NH_4^+ concentration was significantly higher ($p<0.001$) at JC than at the other study sites (SH, OP and DG). In some of the wells at JC and OP e.g., JC2B, JC2C, JC32, JC33 and OP2B, it was remarkably higher than that in other wells. Elevated NH_4^+ concentrations were measured at JC and OP that ranged from 0.05 - 0.22 mg N L^{-1} (Appendix 11). However, NH_4^+ was lower than the EQS value of 0.175 mg N L^{-1} at all sites except at JC where it was higher than the EQS in some occasions mainly in summer. The coefficient of variations over time at individual sites were 257-324, 372-424, 139-301 and 600%, respectively, at JC, SH, OP and DG sites. However, NH_4^+ concentrations were always lower than the value of environmental concern for drinking water (0.23 mg N L^{-1}).

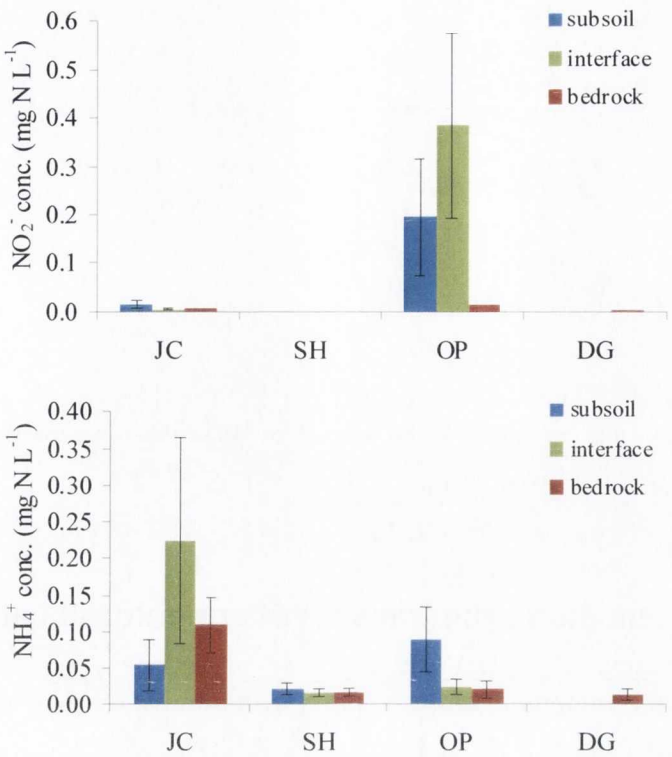


Figure 5.32Groundwater NO_2^- (top) and NH_4^+ (bottom) in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n = 24)

5.5.5.3 Dissolved organic N (DON)

Mean DON concentrations over the two year periods were significantly higher ($p>0.05$) at OP (1.22 mg N L^{-1}) and DG (1.76 mg N L^{-1}) sites than at JC (0.36 mg N L^{-1}) and SH (0.22 mg N L^{-1}) sites (Figure 5.33). However, no significant differences were observed between JC and SH; and between OP and DG. The DON was consistent with the higher permeability where the GWT was comparatively deeper in aquifers dominated with coarse sands and gravels at OP and DG site.

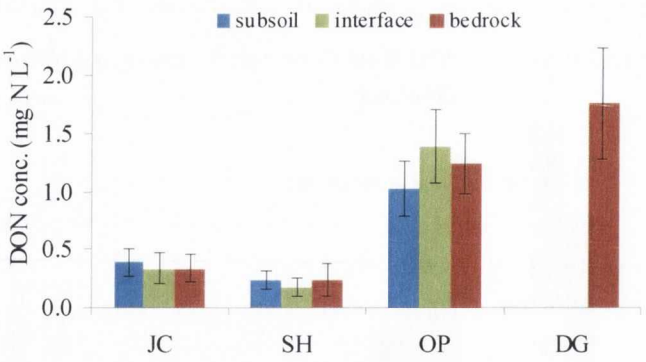


Figure 5.33 Groundwater DON in subsoil, interface and bedrock at (JC) Johnstown castle, (SH) Solohead, (OP) Oak Park and (DG) Dairy Gold; (mean ± SE; n = 24)

5.5.6 Nitrate distributions along the transect of agricultural catchment

Nitrate distributions across the catchment along transect of groundwater flow path showed high spatial variations at all sites. At JC, there was a general trend of decreasing NO_3^- concentrations from the top gradient of the transect towards the down gradient (Figure 5.34) in all wells in all depths except in wells JC3A, JC3B and JC3C, which showed higher concentrations than all other wells, and wells JC2B and JC2C which showed similar concentrations to the down most wells. A very distinct pattern of decreasing NO_3^- concentrations was observed at SH site, where NO_3^- concentrations were highest in the divide of groundwater and lowest in the down most wells (Figure 5.35). NO_3^- distributions at OP site showed opposite pattern to JC and SH sites, where nitrate concentration increased in the down slope of the field along groundwater flow paths (Figure 5.36). NO_3^- distributions in DG site were even more heterogeneous and complex than all other sites, which did not show any definitive patter along the flow paths (Figure 5.37).

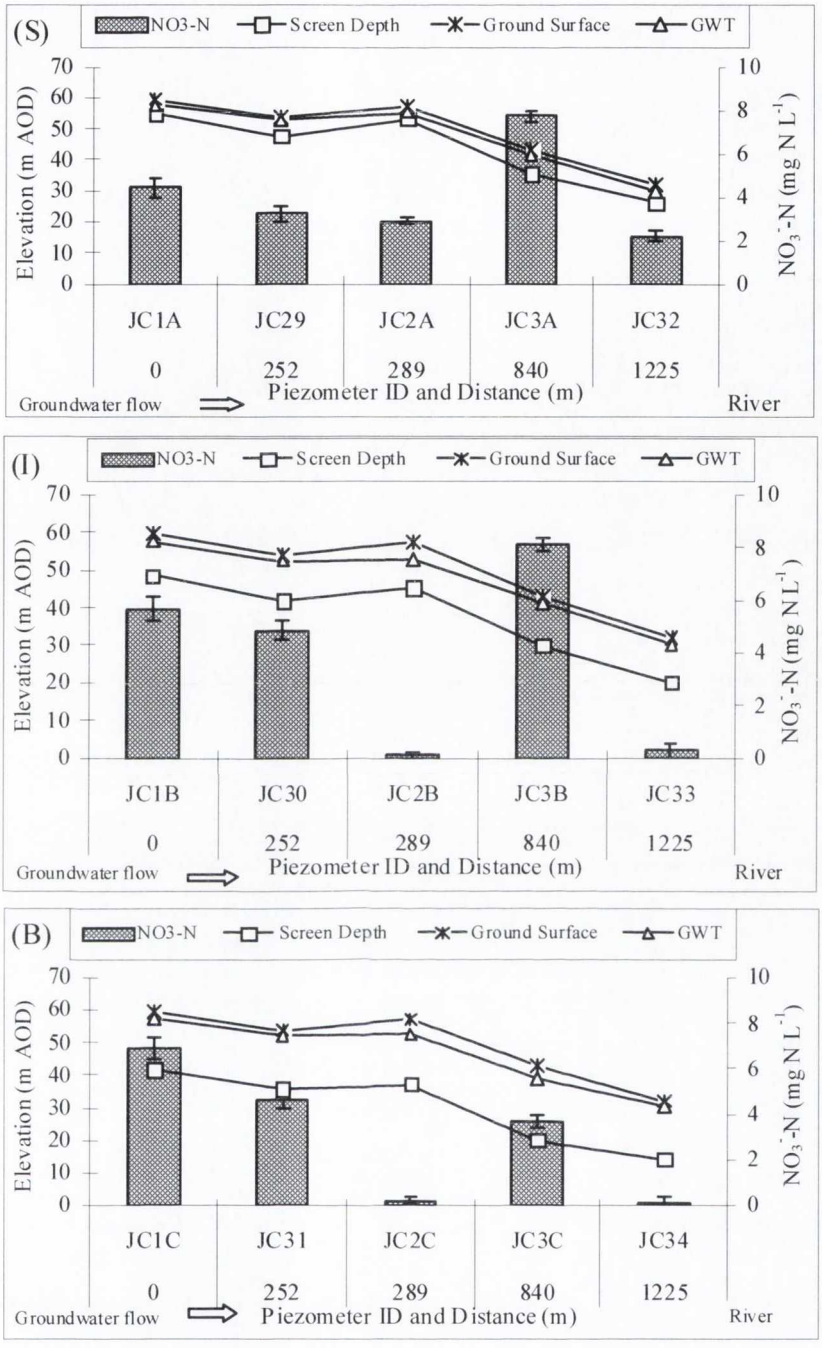


Figure 5.34 Nitrate distributions along the transects at JC in (S) subsoil, (I), interface and (B), bedrock

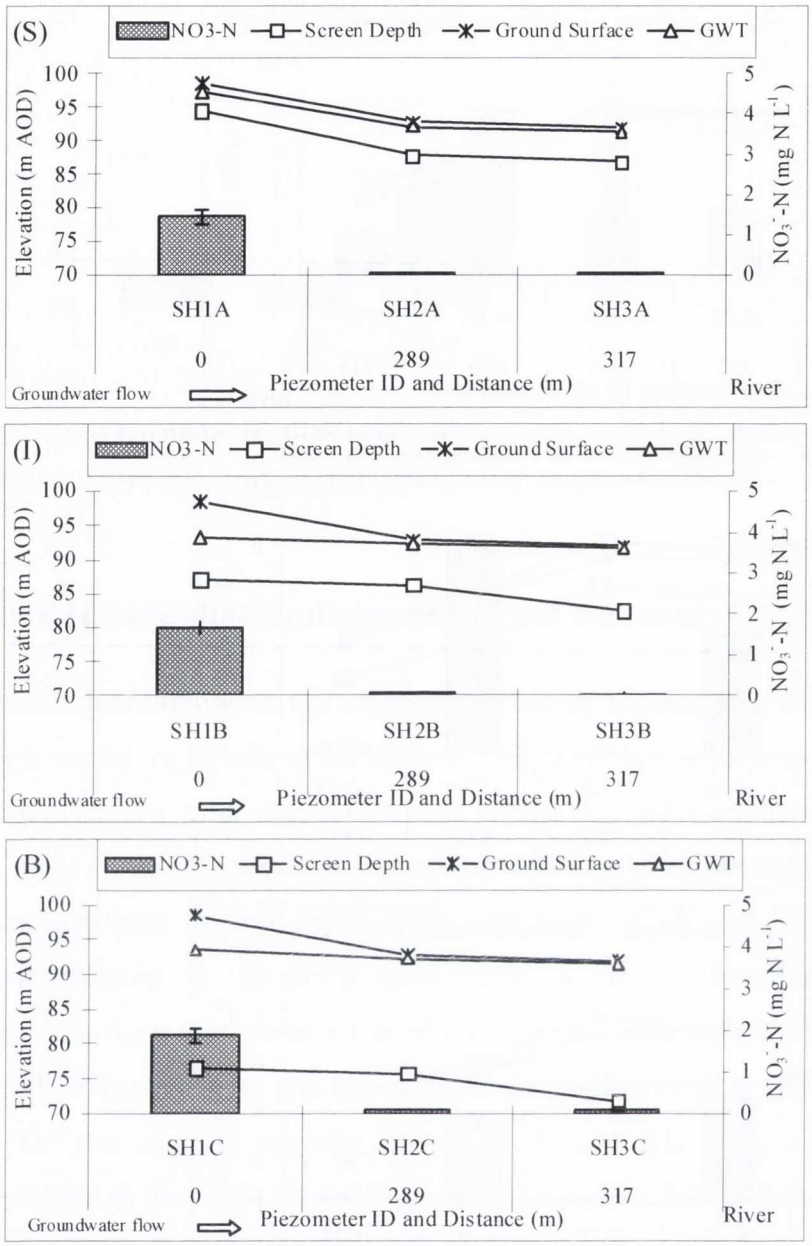


Figure 5.35 Nitrate distributions along the transects at SH in (S) subsoil, (I), interface and (B), bedrock

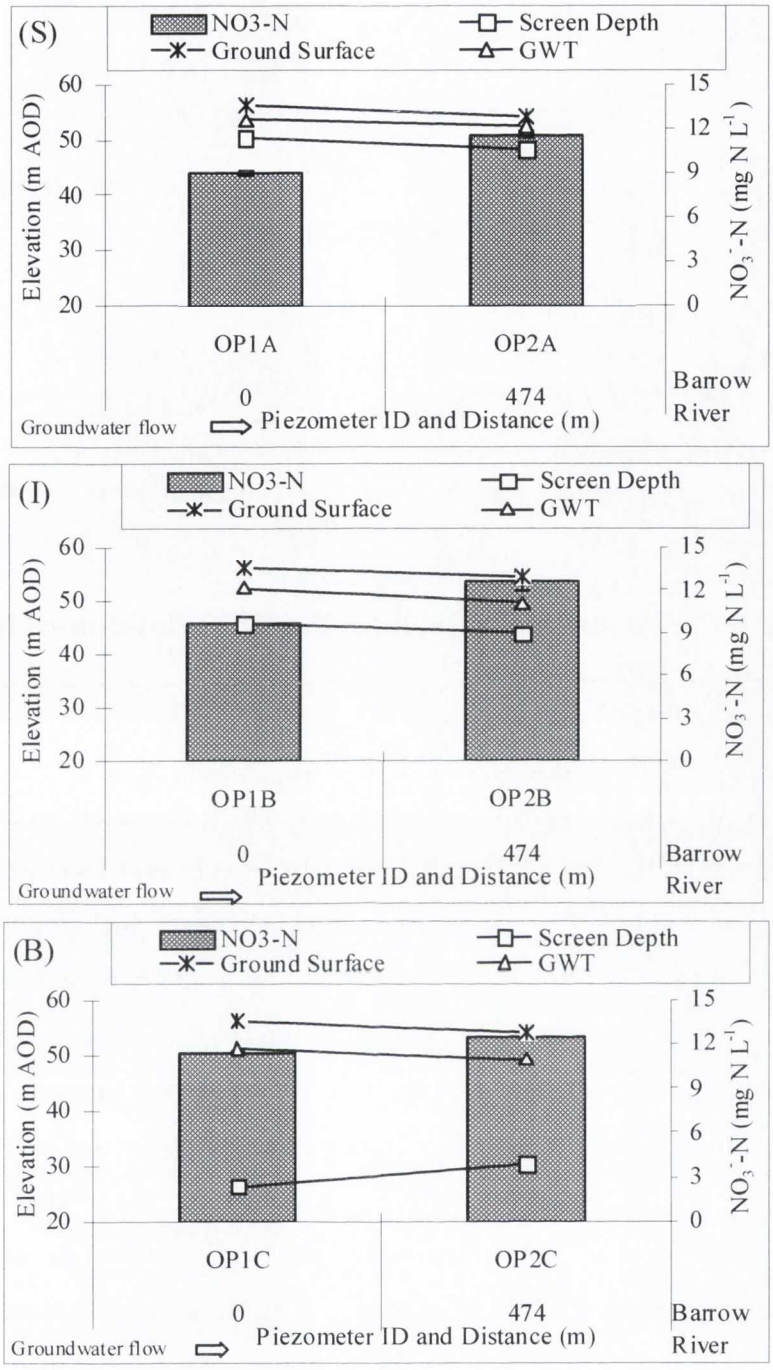


Figure 5.36 Nitrate distributions along the transects at OP in (S) subsoil, (I), interface and (B), bedrock

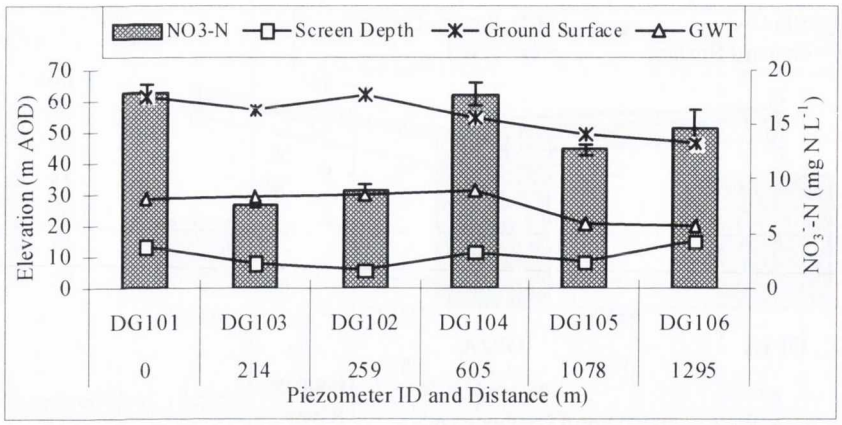


Figure 5.37Nitrate distributions along the transect at DG in (S) subsoil, (I), interface and (B), bedrock

5.5.7 Environmental processes controlling the abundances of NO_3^--N

5.5.7.1 Chloride (Cl^-) to NO_3^- ratio

The pattern of changes in Cl^- concentrations were approximately consistent over time with the coefficients of variation 16, 28, 32, and 43% at JC, SH, OP and DG whereas NO_3^- concentrations showed moderate to high temporal changes. However, the temporal changes in NO_3^- concentrations at OP and DG sites were low (similar to the temporal distributions of Cl^- concentrations over time), being with CV 36 and 44%. Changes in chloride concentrations over time can be attributed to the physical processes such as dilution or advective dispersion, in particular. Conversely, the larger variations in NO_3^- concentrations over the sampling time especially at JC and SH sites than the variations in chloride concentrations suggesting possible occurrence of biogeochemical retention of nitrate in groundwater. In addition, changes in Cl^-/nitrate ratio over time were remarkably high which showed higher amplitude of fluctuations during June to September than that of the other sampling times of the year (Figure 5.38). The Cl^-/nitrate ratio showed even higher fluctuations over time in 2010 when rainfall was lower in Ireland than in 2009.

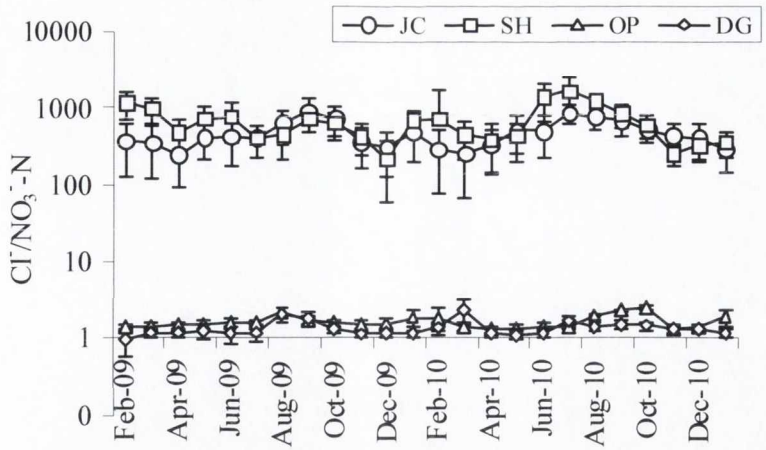


Figure 5.38The fluctuations of chloride/nitrate ratios over time at four different sites: (JC), Johnstown castle, (SH), Solohead, (OP), Oak Park and (DG), Dairy Gold

5.5.8 Linking between NO_3^- and hydrologic and geochemical properties

Plots between NO_3^--N and groundwater hydrogeochemical properties revealed that NO_3^--N concentration in groundwater is a function of ambient hydrogeochemical conditions. NO_3^--N concentrations showed linear and positive correlation with the depths of unsaturated (normalized with the ratio of depth bwt to depth bgl) and permeability of aquifer, K_{sat} (Figure 5.39a and b). Furthermore, NO_3^--N concentrations were positively correlated with DO and Eh (Figure 5.40a and b) which are more likely to be the indicators of groundwater aerobiocity. Conversely, NO_3^--N concentrations showed strong negative correlations with NH_4^+ and CH_4 concentrations (Table 5.2), being an indicator of groundwater anaerobiocity. Decreased NO_3^--N concentrations were observed with the increase in Fe^{2+} and Mn^{2+} concentrations in groundwater (Table 5.2). NO_3^--N concentrations decreased with the increase in SO_4^{2-} concentrations at JC and SH but in OP and DG it showed inverse relation. In addition, SO_4^{2-} concentrations increased in groundwater with corresponding decrease in S^{2-} ions (Table 5.2).

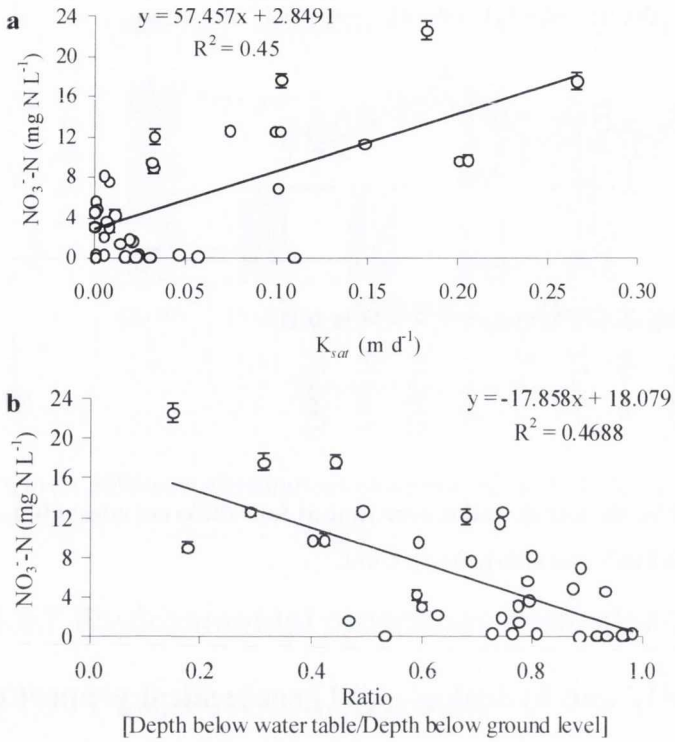


Figure 5.39Plots showing relations between NO_3^--N concentrations and (a) K_{sat} values (n = 36) (a); (b) ratios of depth bwt to depth bgl (n = 36)

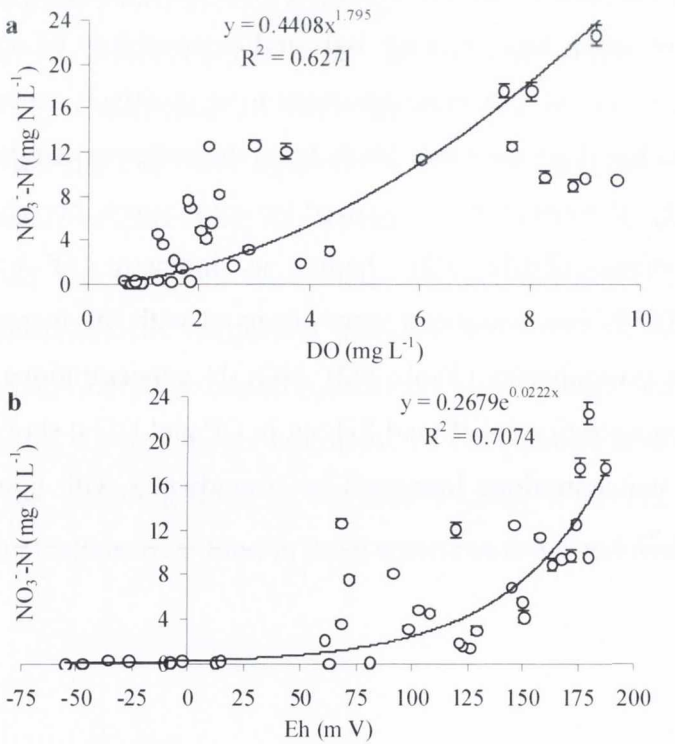


Figure 5.40Plots showing correlations between NO_3^--N concentrations and (a) DO and (b) Eh using the mean data during whole study period (n = 36)

Multiple linear regressions following the stepwise method based on the changes in F-values revealed a good fit model where logDOC, and logCH_4 showed negative relationship and logEh and LogK_{sat} showed significant positive relationship with the predicted NO_3^--N concentrations which could explain 74% of the variances of groundwater ambient NO_3^--N concentrations:

log NO_3^--N = -0.964 - 1.245 logDOC + 0.865 logEh - 0.342logCH4 +0.156logK_{sat} (R^2=0.74; p<0.001; n = 792) (Eqn. 5.10)

where NO_3^--N, DOC, CH_4 concentrations are in mg L^{-1}; Eh is in mV and K_{sat} is in m d^{-1}. The model sequentially included the variables with their relative contributions as shown by F values of 78.22, 50.76, 37.69, and 21.46, respectively for logDOC, logEh, logCH_4, and logK_{sat}. In addition, sampling dates had significant role on excess N_2 concentrations (F value 7.4) in some occasions contributing substantial changes in the intercept of the model (lowest in August-September).

Table 5.2Pearson's correlation co-efficient (r) among groundwater hydrogeochemical properties (n=36)

	NO_3^--N	NO_2^-	NH_4^+	DON	GWT	K_{sat}	Temp.	pH	DOC	CO_2	CH_4	SO_4^{2-}	S^{2-}	DO	Eh	Fe^{2+}	Mn^{2-}
NO_3^--N																	
NO_2^-	0.29*																
NH_4^+	-0.18ns	0.51**															
DON	0.83**	0.22*	-0.02ns														
GWT	0.66**	0.05ns	-0.15ns	0.70**													
K_{sat}	0.46**	-0.02ns	-0.20ns	0.54**	0.63**												
Temp.	0.26*	-0.25*	-0.23*	0.33*	0.55**	0.56**											
pH	0.02ns	0.18ns	0.03ns	-0.05ns	-0.24*	0.15ns	-0.24*										
DOC	-0.31*	-0.20ns	0.25*	-0.03ns	0.00ns	-0.17ns	0.22*	-0.31*									
CO_2	0.04ns	-0.20ns	-0.04ns	0.14ns	0.31*	-0.09ns	0.21*	-0.87**	0.33*								
CH_4	-0.72**	0.15ns	0.44*	-0.54**	-0.56**	-0.49**	-0.32*	0.18ns	0.33*	-0.20ns							
SO_4^{2-}	0.36*	0.20ns	0.14ns	0.56**	0.25*	0.39*	-0.22*	0.13ns	0.13ns	-0.12ns	-0.22*						
S^{2-}	-0.03ns	0.13ns	0.04ns	-0.30*	-0.30*	-0.38*	-0.17ns	-0.04ns	-0.22*	0.01ns	-0.17ns	-0.19ns					
DO	0.80**	-0.06ns	-0.30*	0.66**	0.65**	0.44*	0.38*	0.01ns	-0.06ns	0.09ns	-0.76**	0.19ns	-0.03ns				
Eh	0.79**	-0.06ns	-0.41*	0.61**	0.64**	0.52**	0.40*	-0.20ns	-0.18ns	0.25*	-0.88**	0.17ns	-0.00ns	0.87**			
Fe^{2+}	-0.52**	-0.32*	0.06ns	-0.24*	-0.11ns	0.03ns	0.22*	-0.02ns	0.46**	-0.01ns	0.36*	0.06ns	-0.18ns	-0.32*	-0.43*		
Mn^{2-}	-0.76**	-0.01ns	0.38*	-0.63**	-0.55**	-0.44*	-0.26*	-0.17ns	0.16ns	-0.01ns	0.62**	-0.22*	0.01nsn	-0.73**	-0.68**	0.36*	

ns nonsignificant; * significant at 5% level and ** significant at 1% level

5.5.9 Soiled water irrigation and groundwater NO_3^- reduction

Soiled water collected as milking parlour wash water, milk spillage, runoff from cattle yard areas and effluent from silage and manure were irrigated by surface sprinkler irrigation method over approximately 10 years on a 4 hectare land around the wells JC2A, JC2B and JC2C. The composition of the dirty water used is presented in Table 5.3. Mean TN concentration was 178 kg N ha^{-1} y^{-1}, of which 91% was DON and the rest was NH_4^+-N. There was a high biochemical oxygen demand (BOD) of dirty water (range: 1570-8400 mg L^{-1}; mean 3084 mg L^{-1}) which indicates the presence of C for microbial decomposition. Mean DOC concentrations were 16, 8 and 7.5 mg L^{-1} over the 2- year monitoring periods in JC 2A (5 m bgl), JC2B (12 m bgl) and JC2C (22 m bgl), respectively. Mean TN concentrations were 4.05, 0.80 and 1.07 mg L^{-1}, respectively, in JC2A, JC2B and JC2C. Mean NO_3^--N concentrations were 4.05, 0.09 and 0.38 mg L^{-1}, respectively in JC2A, JC2B and JC2C. Interestingly, DOC, TN and NO_3^--N concentrations were approximately similar in bedrock-interface and bedrock, indicating that in bedrock DOC and TN may be also added via a preferential pathway. Similar DOC concentrations in interface and bedrock also indicated that the fraction of DOC that leached out below subsoil (after substantial consumption in subsoil) may not be bioavailable. Therefore, nitrate reduction in bedrock-interface and bedrock can be attributed to the autotrophic denitrification where other electron donors (Fe/S) were available. The DOC, TN and NO_3^--N profiles along the depths showed that they were abruptly decreased in bedrock-interface and bedrock, suggesting that DOC consumption and denitrification started in shallow groundwater (JC2A) and continued while it was passing vertically downward to deeper groundwaters. However, the TN and NO_3^--N in groundwater beneath the dirty water irrigated field were almost close to the detection limit. The geochemical compositions of groundwater in these three wells (Appendices 9-11) were very favourable for biochemical nitrate reduction (DO and Eh were very low but the DOC was high). These biochemical environments due to dirty water irrigation therefore created a denitrification hot spot in groundwater, resulting in a nitrate free groundwater.

Table 5.3Mean concentrations of N and other constituents of soiled water sprayed at JC around the wells JC2A, JC2B and JC2C

Constituents	Concentrations (mg L^{-1})	Volume of water* irrigated (L y^{-1})	Total amount (kg ha^{-1} y^{-1})
TN	351	506250	178
NH_4^+-N	32		16
TON	0.3		0.2
NO_2^--N	0.3		0.2
DON	318.7		161
TP	44		22
K	415		210

Sources: Water Lab, Johnstown Castle Environmental Research Centre;
*estimated based on the information from Aidan Lawless, farm manager, JC dairy farm

5.5.10 Interpretation of the Results

5.5.10.1 Implication of hydrology on the abundances of NO_3^-

The GWT fluctuations reflect the pattern of rainwater recharge and drainage to and from groundwater, which had a significant implication on groundwater hydrochemistry. It showed the change in the depth of unsaturated zone overlying the saturated zone over the sampling period. The deeper GWT significantly increased groundwater DO and Eh (Table 5.2) and hence reduced the nitrate retention capacity because, NO_3^--N reduction follows the DO consumptions (Puckett and Cowdery, 2002; Thayalakumaran et al., 2008). At DG and OP, deeper unsaturated zones, with correspondingly higher K_{sat} values, revealed higher solute transport potential i.e. higher vulnerability than JC and SH. One crucial hydrologic insight on the groundwater geochemistry is that DO and Eh significantly increased with groundwater recharge when depth of unsaturated zone is higher (approximately greater than 5 m). Because DO at the OP and DG sites was 1.48 and 1.54 mg L^{-1} higher in 2009 (ER 537 and 684 mm) than in 2010 (ER 241 and 336 mm), whereas at JC and SH sites increase in DO in 2009 from 2010 was negligible (0.2 mg L^{-1}). In 2009 ER was higher (Chapter 5) than 2010 with correspondingly higher NO_3^--N concentrations (3.9, 1.0, 11.1 and 14.9 mg N L^{-1} in 2009 and 3.4, 0.6, 10.9 and 14.3 mg N L^{-1} in 2010), suggesting higher NO_3^--N flushing from soil with high ER together with lower biogeochemical reduction in 2009, may be due to also flushing higher DO. These results confirm the reverse situations in 2010 with lower NO_3^--N coupled to lower DO. Therefore, it is obvious that nitrate response to hydrology is not only due to physical process but it also implies the

impact of hydrology on groundwater biogeochemistry. Bartley and Johnston (2006) argued that groundwater NO_3^--N response is hydrologically related. Higher NO_3^--N concentrations were consistent with higher K_{sat} (approximately >0.05 m d^{-1}) values suggesting that groundwater travel time is inversely related to groundwater NO_3^--N reduction. Because higher K_{sat} results from the numerous larger as well connected pores which enriched groundwater with DO that equilibrated with infiltrating water. Therefore, larger K_{sat} with correspondingly deeper GWT have significantly high potential of groundwater NO_3^--N concentrations which were observed at OP and DG, in particular. Because sediments with coarse sands allow faster leaching through larger as well as better connected pores (Goss et al., 1998) which shows higher groundwater vulnerability (NRA, 1995) than clayey soils. The DO in groundwater can be consumed by DOC that produces CO_2. However, DOC input was not sufficient in groundwater to significantly consume DO at OP and DG sites. As a consequence of free draining conditions, DO concentrations eventually increased in groundwater which ultimately affected the overall biogeochemical N transformation. Conversely, the shallow unsaturated zone at JC and SH corresponded to lower permeability but higher NO_3^- residence time and higher nitrate removal capacity than DG and OP. Tsushima et al. (2002) stated that groundwater with low DO had an over five-fold longer residence time. In addition, slower permeability increases the potential to build up shallower GWT and can lower unsaturated area. Young and Briggs (2007) concluded that shallow GWT would favour greater denitrification and lower leaching potential. High permeabilities with the correspondingly high thickness of unsaturated zone are also consistent with the high Eh. The GWT is known to play a regulatory role in the functioning of shallow groundwater ecosystems by supplying organic matter for heterotrophic metabolism (Baker et al., 2000). Therefore, deeper GWT being with deeper unsaturated zone and higher K_{sat} are not favourable for biochemical nitrate reduction which in turn increased the vulnerability of groundwater to nitrate. This argument can be supported by the significantly higher DON concentrations at OP and DG sites than JC and SH. Because of the higher permeability at OP and DG, higher amount of DON escaped from further transformations in unsaturated zone and as such directly reached groundwater.

5.5.11 Hydrogeochemistry and the abundances of NO_3^-

5.5.11.1 Temperature

Groundwater temperature was similar across sites. It changed over time in subsoil and interface whereas bedrock did not respond to temperature changes in the atmosphere. A similar range of groundwater temperature (11-13°C) to this study was reported by Bartley and Johnston (2006), being measured at Curtin's Farm, Moore Park during 2000-2003. They also measured the lowest temperature in February and March and highest in summer and autumn. Temperature has an important role in biogeochemical reactions in any environment. Similar temperature in bedrock over the year may have good implications on minimising dramatic changes in groundwater hydrogeochemical transformations. Nonetheless, even though reports on the relations between temperature and nitrate in groundwater are very scarce, a very weak negative correlation was observed between the abundances of nitrate and temperature in our study. Lower nitrate with higher temperature can be because of the higher denitrification in groundwater in higher temperature. Pfenning and McMahon (1997) explained that only 15–30% of nitrate in groundwater was denitrified before discharging to the South Platte River and that nitrate concentrations in the river were generally higher in winter (low temperature) than in summer (high temperature). They summarized that lowering incubation temperatures from 22 to 4°C resulted in about a 77% decrease in the N_2O production rates in groundwater. Kellogg et al (2005) measured higher denitrification rates in subsoil with a higher temperature and showed significant reduction in nitrate concentrations. Saunders and Kalff (2001) observed that a 5°C increase resulted in a 10-fold increase in denitrification rate. According to previous research, overall temperature ranges across sites can not be assumed to be inhibitory to denitrification, as Tsushima et al. (2002) commented that a groundwater temperature range of 11.8 to 13.9 would be reasonably active for denitrification to occur. Robertson et al. (2000) demonstrated a correlation between water temperature and denitrification rates in a permeable reactive barrier system. They summarized that denitrification was observed down to 2°C; between 2 and 5°C, rates were approximately 5 mg N $L^{-1}d^{-1}$and; between 10 and 20°C, rates increased to 15–30 mg N $L^{-1}d^{-1}$.

5.5.11.2 pH

pH was near neutral but different across sites, suggesting that different biogeochemical conditions are prevailing in each site. Exceptionally high pH (>9.0) at OP site was due to the presence of calcareous materials. The pH value complied with the EPA IGV pH range of 6.5 to 9.5. No extremely low pH was observed at any site, indicating that nitrate biogeochemical transformation will not be inhibited if other conditions are favourable. Thayalakumaran et al. (2008) reported similar results for groundwater pH in Burdekin, Australia, as they noted that groundwater was mostly neutral to alkaline with no obvious spatial and temporal variability observed. However, 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). Generally, increasing pH between 5.5 and 8.0 increases denitrification rates (Rust et al., 2000) and decreases nitrate concentrations, but in this case, pH did not seem to be an important controlling factor as pH in these groundwaters across sites were very suitable, unlike interface at OP, for denitrification to occur. However, the optimal pH is site-specific because of the effects of acclimation and adaptation on the microbial ecosystem (Rust et al., 2000).

5.5.11.3 Dissolved Organic C (DOC)

Groundwater DOC concentrations were very small in amount at all sites (mean DOC 0.90 mg L^{-1} in OP to 2.92 mg L^{-1} at JC), but their similar concentrations in all depths indicates that DOC can leach out from surface soil to deeper groundwaters that can affect groundwater biogeochemistry. However, a consistently lower DOC was observed in bedrock suggesting that DOC was consumed in subsoil and interface zone while passing downward to the bedrock zone. Chemical transformation and sorption processes resulted in a constant decrease of instable organic matter with depth (Siemens et al., 2003; Jorgensen et al., 2004). Generally DOC concentrations in most aquifers are relatively low, typically <5 mg L^{-1} (Rivett et al., 2007). However, Puckett and Cowdery (2002) have shown that even small amount of organic C can support denitrification and can cause measurable decrease in NO_3^- concentrations in groundwater. A negative correlation between DOC and NO_3^- indicated the occurrence of denitrification in groundwater and supported the stoichiometry of 1:1.25 ratio of NO_3^--N to DOC (Korom, 1982). Moreover, DOC can

consume DO and produce CO_2 which can be reduced to CH_4. Consumption of DO by DOC indirectly affects nitrate concentrations as it creates anaerobic environments for microbial reduction of NO_3^--N to N_2. Substantial CO_2 reduction to CH_4 was observed in approximately 35 and 60% of the wells at JC and SH which wells are indicating the existence of anaerobic environment for NO_3^- reduction. Furthermore, DOC was negatively correlated to Eh, suggesting that DOC enhances denitrification by enhancing anaerobic conditions in groundwater. Brettar et al. (2002) reported a decrease in redox potential with the increase in organic matter content in floodplain soils which was consistent with potentially low nitrate concentrations. Similar concentrations of DOC to these study sites were reported by Starr and Gillham (1993), Wassenaar (1995), Beller et al. (2004) and Mohamed et al. (2003); and higher than these sites by Thayalakumaran et al. (2008). Higher spatial variability in DOC concentration indicates the higher variability in water percolation, leaching, land topography, management practices etc. In JC2A, JC2B and JC2C, respectively in subsoil, interface and bedrock unusually high DOC concentrations (8-25 mg L^{-1}) were measured which was accumulated due to dirty water irrigation (Table 5.3) over the last couple of years (approximately 10 years) which influenced the DO, Eh and other biogeochemical variables like NO_3^--N and SO_4^{2-} concentrations (Appendices 9-11). Higher spatial variability of DOC was in line with von der Heide et al. (2008) who found 68% CV of DOC in shallow groundwater in sandy aquifer in Germany. It appears that DOC at JC and SH sites, being with low nitrate concentration, can be an important electron donor even though it is not sufficient to reduce substantial amount of nitrate. However, at OP and DG sites DOC appears not to be an important source of energy for microbial denitrification. Nonetheless, it can be concluded that DOC seems to be a limiting factor in groundwater nitrate reduction in these study sites, where other electron donors can act as electron sources for biological nitrate reduction.

5.5.11.4 Dissolved CO_2 and CH_4

Despite the low DOC concentrations, dissolved CO_2 and CH_4 concentrations show that groundwater can be an important storage for C sequestration as well as atmospheric CO_2 and CH_4 emissions upon discharge to the surface waters. Dissolved CO_2 in river water is an important component of the terrestrial carbon cycle and an important pathway for CO_2 emissions to the atmosphere (Minamikawa et al., 2010). The C balance for net ecosystems

productivity (NEP) is difficult due to ignoring the dissolved C in groundwater which is now emphasized to measure and to be included in the C balance. Carbon budget for terrestrial ecosystems could be incomplete and net C sequestration could be overestimated if hydrologic export of evaded CO_2 together with other dissolved C are not considered (Tarnvik et al., 2009; Cole et al., 2007). Upon discharging to the receptors, CO_2 and CH_4 can be degassed to the atmosphere that eventually contribute to the global warming as they are important greenhouse gases. Dissolved CO_2 and CH_4 concentrations in groundwater have recently been reported by few other researchers. Groundwater acts as a pathway for indirect greenhouse gases (GHGs) emissions and a medium where they are produced and consumed (Minamikawa et al., 2010). As groundwater enters streams and rivers, much of the GHGs are rapidly emitted to the atmosphere, thus transferring C and N, fixed in terrestrial ecosystems, to the atmosphere via aquatic pathway. Worrall and Lancaster (2005) reported that groundwater in the UK was an important source of dissolved CO_2 in the rivers. Johnson et al. (2006) found that stream flow in the Amazonian headwater catchment was predominantly derived from deeper flow paths containing water with high dissolved CO_2 concentrations.

Gooddy and Darling (2005) reported, from groundwater in four major UK aquifers, that high CH_4 concentrations were observed where groundwater had the highest reductive potential. The concentration gradient of dissolved CH_4 between ambient air and water bodies will cause a substantial amount of CH_4 emissions from groundwater to the atmosphere (Sawamoto et al., 2003). Substantial amount of CH_4 with $\delta^{13}C$ (-72.1±6.8‰) and $\delta^{2}H$ (-297±17‰) from biogenic origin predominantly from CO_2 reduction was reported by Cheung et al. (2010) from shallow groundwater wells in Alberta, Canada.

5.5.11.5 Available sulphate (SO_4^{2-})

Böhlke (2002) suggests that SO_4^{2-} is a useful tracer for agriculturally impacted groundwater. Similar concentration of SO_4^{2-} in groundwater across sites could be due to two reasons: firstly sulphide reduction to SO_4^{2-} under suboxic conditions (limited DO; <2 mg L^{-1}) resulting in very low nitrate (electron acceptor; 0.7 to 3.7 mg NO_3^--N L^{-1}) as nitrate may have reduced via microbial denitrification (Korom, 1992; Sierra-Alvarez et al., 2007); another one is that sulphur oxidation at high DO concentrations suggesting possibility of

DO consumption by sulphur oxidation. For example, in subsoil at OP higher SO_4^{2-} concentration could be due to sulphide (e.g., pyrite, H_2S, S_2O_3) oxidation under higher DO concentration and higher pH. Inversely, higher SO_4^{2-} concentrations in few wells at JC and SH (JC1A, JC2B, JC2C, JC3A, JC3B, JC29, JC30, JC31; SH2B, SH2C, SH3B, SH3C) could be due to their highly reduced environment (very low DO and Eh) where S^{2-} oxidation might have occurred by NO_3^--N reduction due to absence of DO. The spatial and temporal variability of SO_4^{2-} were similar to NO_3^--N concentrations at all sites and depths. High spatial distributions of SO_4^{2-} in groundwater with CV 86% were found by von der Heide et al. (2008). Groundwater in these study area have no concern for sulphate pollution as they have lower SO_4^{2-} concentrations than the EQS value of 187.5 mg L^{-1} (Craig and Daly, 2010).

5.5.12 Groundwater redox chemistry

Groundwater DO was 0.3 - 10 mg L^{-1} across all sites and depths while at JC and SH sites it was <2.0 mg L^{-1}. Similar DO in groundwater was reported by Beller et al. (2004) in a range of unconfined (4 - 10 mg L^{-1}) to confined (<1 - 4 mg L^{-1}) aquifers in California, USA where denitrification is considered as an important process of nitrate reduction in groundwater. In subsoil, lower DO at JC and SH than OP could be due to their shallow GWT, lower amplitude of GWT fluctuation and lower K_{sat} values. Consumption of DO due to microbial transformations of C to CO_2 could be another reason of low DO in groundwater. Similarly, considering differences in DO in bedrock between 4 sites, higher DO at DG site could be due to deeper unsaturated zone and higher K_{sat} values allowing better aeration. The DO did not vary among depths at each site probably because of hydrogeological heterogeneity e.g. preferential passage both in vertical and horizontal directions. In some wells, DO was higher in subsoil but in some others it was higher at interface or in bedrock (e.g., Appendix 9) which is supported by their remarkably higher CV values. Lower Eh at JC and SH even negative values in some wells may be due to the lower DO concentrations which indicates a potentially reduced environment. Higher DO and Eh during winter (November-January) could be due to the prevailing low temperature in this period, whereas inverse conditions were observed during summer. Another reason of higher DO during this period could be due to DO enrichment with recharging water

from rainfall. In a recently infiltrated recharge water, groundwater becomes fully oxygenated which requires an indicative concentrations of DOC below which anaerobic conditions may not develop (Rivett et al., 2008). The wells that have reduced environment, Fe^{2+} and Mn^{2+} concentrations start to increase but S^{2-} starts to decrease while groundwater Eh drops below 150 mV indicating that the Eh value of 150 mV is the turning point in groundwater when nitrate starts to reduce. Moreover, Fe^{2+} and Mn^{2+} reach their maximum levels in some wells at JC and SH sites when the Eh drops below 100 mV indicating that at ±100 mV nitrate reduction is high. Jaffrés (2004) reported negative correlation between nitrate and Fe^{2+} ions and commented that this negative correlation supports the biological denitrification.

5.5.13 Groundwater N dynamics

The EPA Interim Guideline Value (IGV) (5.6 mg N L^{-1}; EPA 2003) exceeded in each piezometer at OP and DG sites, whereas at JC and SH sites it was lower than the EPA IGV. Very low NO_3^--N concentrations in suboxic groundwater has been observed in a number of other studies, and in some case it has been linked to denitrification in the anoxic zones (Robertson et al., 1996; Tesoriero et al., 2000). Therefore, lower NO_3^--N at JC and SH than at OP and DG indicated that denitrification in groundwater required proper anaerobiocity e.g. DO <2.0 mg L^{-1}, Eh <150 and an electron donor like DOC or reduced Fe/S compounds. Conversely, very small amount of NO_3^--N can be retained probably as N_2O in high DO (mean 6.5 - 8.7 mg L^{-1}) and Eh (100-250 mV) with available electron donors (mainly DOC) and sometimes with reduced S and Fe at OP and DG sites. Unusually, high pH (mean 7.4 - 8.6) at OP could be another reason of low denitrification because Rust et al. (2000) quoted an acceptable higher limit for pH of 8.3 above which denitrification is arrested. The decrease in NO_3^--N concentrations with depths indicated that denitrification can take place along groundwater flow paths from its sources to the receptors (Konrad, 2007) and it is not really confined in shallow layer only. Considering the temporal pattern, similar pattern of NO_3^--N removal in riparian groundwater was observed by Maître et al. (2003), who found highest nitrate removal in spring and lowest in winter due to the combination of a high nitrogen input and a low plant uptake. Thayalakumaran et al. (2008) reported higher NO_3^--N in groundwater during January and

lower during September/October. Brooks et al. (1998) and Sickman et al. (2003) reported that stream nitrate concentrations typically peak just before or at the peak of spring snowmelt, declined throughout the summer, and rise slightly over the winter. This is caused by relative differences in the rates of nitrification, denitrification, microbial immobilization, and plant uptake. They also reported the dominance of denitrification in the late spring, and then microbial immobilization becomes more important in summer. However, the spatial variability was more pronounced than temporal indicating that groundwater hydrochemical properties are complex and heterogeneous.

The NO_2^--N and NH_4^+ concentrations were almost absent in either of the groundwater zones except few wells at OP (OP2A and OP2B) which have considerably higher NO_2^--N and few wells at OP (OP1A and OP2A) and JC (JC2B, JC2C, JC33 and JC34) which have considerably higher NH_4^+ during the study period. The NO_2^- at OP exceeded the EPA IGV of 0.03 mg N L^{-1} (EPA, 2003) but this may not create a threat to groundwater NO_2^- pollution because it is an intermediary constituent of N cycle, which will convert to NO_3^- at favourable environments, as was seen in bedrock zone there was no NO_2^-. The EPA IGV for NH_4^+ is 0.12 mg N L^{-1} (EPA, 2003) which was exceeded in several wells at JC and OP which persistently breached the EPA IGV. The wells (JC2B, JC2C, JC33 and JC34) at JC consistently breached the Drinking Water Regulations parametric (MAC) limit of 0.23 mg NH_4^+-N L^{-1} (DELG, 2000) and EQS of 0.065 mg N L^{-1}. This intermediary accumulation can be due to the interruption in nitrification/denitrification by high soil pH (>8.5), because pH range above this range can cause NO_2^- accumulation in presence of high nitrate concentration (Glass and Silverstein, 1998). Shen et al. (2003) concluded from an N dynamic study under different pH that nitrite was unstable in acid soils, but durable in alkaline soils (pH >7.89). Temporal changes in NO_2^--N concentration shows approximately consistent trend with slightly higher in winter across all sites and depths because of higher leaching potential with rainwater or could be due to lower chemical and microbial changes during this period. Relatively constant NO_2^--N concentrations were also reported by Beller et al. (2004) in denitrifying aquifer in USA. A steady decline in groundwater NO_2^--N was also reported by Brodie et al. (1984). Spatial variability of NO_2^--N concentration was rather higher in groundwater than soil and resembles to the higher spatial structure of groundwater biogeochemical variables which implies that groundwater nitrate is not a conservative ion rather it undergoes biogeochemical changes in groundwater while passing

through and from landscape to potential receptors. Similar spatial variability of NO_2^--N in groundwater (CV 24%) was reported by von der Heide et al. (2008). Nitrate showed negative correlation with NH_4^+, indicating dissimilatory nitrate reduction to ammonium (DNRA). Ammonium is the end product of DNRA which is a process that can temporarily remove nitrate (Lamontagne et al., 2003; Tesoriero et al., 2000). Higher permeability can increase DON in groundwater due to the lower residence time for DON transformations to nitrate. Groffman et al. (1993) suggested that in the well drained landscape there was a large amount of microbial immobilization, as a result there was little nitrification or denitrification. In the poorly drained soils may be there have been little microbial immobilization; as a result NH_4^+ availability to nitrifiers was high and rates of nitrification was high that fostered high denitrification N losses.

5.5.14 Nitrate distribution along groundwater flow paths

There was a decreasing pattern of nitrate distributions along transect of groundwater flow paths except some specific wells at JC (Figure 6.18) which have different management history that results in different hydrogeochemical environments. Exceptional nitrate distributions in these wells were due to the variations in local management practices and hydrogeochemical environments e.g., plots around the wells JC2B and JC2C have been under dirty water irrigation for about 10 years which increased the DOC concentrations of the wells (Appendix 9). At JC 3A, JC3B and JC3C, there were high DO and Eh and low DOC which were not favourable for microbial denitrification. The decreasing pattern of nitrate distribution from top to the down gradient of the transect suggesting the potential for biogeochemical nitrate removal i.e., the longer is the residence time of groundwater along its flow path the higher is the nitrate retention. The most indicative wells were JC32, JC33 and JC34, which have the lowest nitrate concentrations. This can be attributed to the nitrate retention while passing from the top gradient towards the down gradient and favourable hydrogeochemical conditions in the down gradient where DO, Eh, and K_{sat} were lower and GWT were shallower than the top gradient wells. The increasing concentrations of nitrate at OP site along the flow paths, suggesting the enrichment of nitrate, while groundwater was passing through and from the landscape. It can be attributed to the local hydrogeochemical conditions (Appendices 9-11) where groundwater

hydrogeochemical environments were not suitable for denitrification to occur. There was no distinctive pattern of NO_3^- distributions at DG site, which can be due to the high spatial variations in groundwater hydrogeochemical conditions (Appendix 11), multiple directions of groundwater flow, karstification which may have the bypass flow and caves (Landig, 2009).

5.5.15 NO_3^- reduction processes and factors

Neither chloride nor nitrate is affected by chemical processes in groundwater except where nitrate may undergo denitrification (Buss et al., 2005) and an increase in the Cl^-/ NO_3^- ratio indicates that NO_3^- removal process e.g., denitrification occurs (Altman and Parizek, 1995; Mengis et al., 1999). This compensates for changes in nitrate concentration caused by mixing of groundwater with different composition. Nitrate concentration decreases resulting in the increase in Cl^-/nitrate ratio potentially suggesting that nitrate reduction is not only a function of dilution but also a process of denitrification. Van Beek et al. (2007) found that the increase in Cl^-/ NO_3^- ratio in groundwater was due to nitrate removal by denitrification which was supported with the observed changes in groundwater $\delta^{15}N$.

The DO correspondingly increases the Eh. Positive correlation between NO_3^--N and DO and Eh (Figure 6.24a and b) indicates that low NO_3^--N in groundwater with low DO and Eh is due mainly to denitrification because low DO and Eh favour denitrification process (Thayalakumaran et al., 2008). At the JC and SH sites, mean DO (mean 1.7 and 1.4 mg L^{-1}) and Eh (71 and 60 mV) indicated the potential of those sites for denitrification to occur. DO concentration <2 mg L^{-1} and Eh values <250 mV have been reported to be favourable for denitrification (Korom, 1992). But, systems seldom exhibit strict redox zone boundaries as a number of redox reaction may occur simultaneously in any single aquifer block (McGuire et al., 2000). Therefore, 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). Low DO and Eh, and availability of electron donors are used as geochemical indicators to indicate conditions suitable for groundwater denitrification (Thayalakumaran et al., 2008). Rivett et al. (2008) identified O_2 and electron

donor concentration and availability as the primary factors governing denitrification in groundwater.

Ammonium production in groundwater is an indication of the anaerobic conditions which shows significant negative correlation with NO_3^--N, indicating that NO_3^--N reduction occurs in groundwater in an anaerobic environment. Negative correlations of groundwater NH_4^+ concentrations with NO_3^--N, DO and Eh imply that both DNRA and denitrification take place in groundwater at anaerobic conditions. High ammonium concentrations in few wells showed low NO_3^--N concentrations indicating the existence of DNRA because low NO_3^- with high level of NH_4^+ suggests the occurrence of DNRA (Thayalakumaran et al., 2008). DNRA is an anaerobic process where NO_3^- is transformed to NH_4^+ (Tesoriero et al., 2000). DNRA is favoured in NO_3^- limiting conditions (Korom, 1992; Kelso et al., 1997). Similarly, CH_4 production in groundwater shows the anaerobiocity in groundwater which in turn shows significant negative relation with NO_3^--N.

Contribution of DOC as electron donor in groundwater denitrification seems to be an important electron donor because it showed significant negative relation with NO_3^--N ($r=-0.317$; $p=0.023$). Denitrification reactions at some sites may be driven by multiple electron donors, for example, where organic carbon, sulphide and iron minerals are coupled (Rivett et al., 2008). However, at all sites the DOC remains relatively consistent over time which indicates that DOC is not completely bioavailable (Siemens et al., 2003) and addition and transformation of bioavailable fractions of DOC in groundwater equates to each other. Korom (1992) suggested that DOC should be more than NO_3^--N to affect denitrification which was observed in some of the wells at JC and SH. In contrast, Beller et al. (2004) reported that 0.7 - 1.3 mg L^{-1} DOC in NO_3^--N contaminated aquifer (9.5 - 22 mg L^{-1}) is insufficient to meet the electron donor requirements for complete denitrification. A significant positive correlation with DOC and CO_2 was observed (Table 5.2). In denitrification process, if organic C is the electron donor, bicarbonate and CO_2 are formed but if reduced S is the electron donor, SO_4^{2-} are formed (Rivett et al., 2008). DOC is first oxidized by DO: this requires 1 mg C L^{-1} to convert 2.7 mg DO L^{-1}. Furthermore, some other particulate C sources can affect denitrification which are not analysed in present study. The stoichiometric reaction can be shown as below:

$$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O \quad \text{(Eqn. 5.11)}$$

$$2CH_2O + NO_3^- + 2H^+ \rightarrow NH_4^+ + 2CO_2 + H_2O \quad \text{(Eqn. 5.12)}$$

$$CO_2 + 8H^+ \rightarrow CH_4 + 2H_2O \quad \text{(Eqn. 5.13)}$$

Both heterotrophic and autotrophic denitrifications were prevailing at groundwater zones because low NO_3^--N concentration was coupled with high DOC and SO_4^{2-} concentrations. Groundwater increased SO_4^{2-} concentrations coupled with low NO_3^--N concentration could be due to sulphide oxidation where S^{2-} (reduced S or metal bound S) might be an important electron donor (autotrophic denitrification). Postma et al. (1991) 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. Kölle et al. (1985) and Weymann et al. (2010) postulated that high NO_3^- removal in the autotrophic denitrification zone is most likely caused by practically anoxic conditions and high reactive microcrystalline pyrite components. The stoichiometric reaction can be shown below:

$$5FeS_2 + 14NO_3^- + 4H^+ \rightarrow 7N_2 + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O \quad \text{(Eqn. 5.14)}$$

Therefore, NO_3^- reduction by iron sulphide or manganese sulphide can release Fe^{2+} and Mn^{2+} ions which were observed in 35% of the wells under study. Numerous researchers have suggested autotrophic denitrification with Fe^{2+}/S^{2-} (Bottcher et al., 1990; Postma et al., 1991; Tesoriero et al., 2000; Weymann et al., 2010). 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; Broers, 2004).

Analysis of groundwater samples for the abundance of denitrifier functional genes in the same wells in our study sites in May and June, 2009 was performed in the Lab of Microbial Ecology, NUI Galway (Data presented in Chapter 6). The denitrification functional genes were present in all sites and depths in similar concentrations ($p>0.05$). The abundance of denitrifying community is generally assumed to be ubiquitous and the denitrifying genes are reported to be widespread in phylogenetically distant organisms (Linne von Berg and Bothe, 1992), in surface water, soil and groundwater (Beauchamp et al., 1989), at great depths in aquifers: in clayey sands to 289m (Francis et al., 1989), in

limestone to 185 m (Morris et al., 1988), and in granite to 450 m depth (Neilsen et al., 2006) but their expressions required favourable environmental conditions.

5.5.16 Conclusions

Groundwater systems have the potential for the natural NO_3^- reduction (via biogeochemical processes) but it shows a large variability between different agricultural sites due mainly to their complex hydrologic (e.g. K_{sat}, changes in GWT depth etc.) and hydrogeochemical (redox chemistry i.e. DO and Eh; DOC and other electron donors like reduced Fe and S, NO_3^- concentration, pH etc.) variabilities. A shallow unsaturated zone with low permeability can substantially reduce nitrate delivery from top soil and unsaturated zone to groundwater and to surface waters. In contrast, high permeability subsoils and glacial till, particularly in limestone aquifer, can be potentially vulnerable to NO_3^- pollution. Though DOC concentration is not sufficient in groundwater for complete denitrification to occur, multiple electron donors together with DOC (metal bound S or sulphide), were available across all the study sites, but hydrogeochemical conditions restricted the extent of NO_3^- reduction in some sites. The DOC, Eh and K_{sat} and DO seem to be the main factors that control the NO_3^- pollution in groundwater. Therefore, the spatial and temporal distributions of different hydrogeochemical properties e.g., DO, Eh, depth of unsaturated zone and K_{sat} can be an important prediction of groundwater vulnerability for nitrate. Nitrate distribution along transect of the field shows generally lower NO_3^- near the stream than the top gradient, suggesting natural attenuation along groundwater flow paths. Mapping up of such properties across the country's variable landscape settings can be an important tool for managing agricultural catchment to reduce the risk for NO_3^- delivery to the environment. Hydrochemical results in few wells at JC shows that soiled water irrigation practices can create a denitrification 'hot spot' by adding substantial amount of DOC in groundwater causing 100% reduction of delivered NO_3^-. Groundwater hydrogeochemistry data are log-normally distributed and more spatially heterogeneous than temporal changes.

CHAPTER 6. IN SITU MEASUREMENTS OF DENITRIFICATION AND DNRA

6.1 Overview of this chapter

In situ monitoring of N_2O and N_2 concentrations in groundwater at four agricultural sites between Feb, 2009 and Jan, 2011 was conducted on a monthly basis and reported in this chapter. In addition, in situ denitrification capacity rates were measured at two sites (Johnstown Castle, JC and Oak Park, OP) those have marked contrast in context with soil type, drainage and groundwater hydrogeochemistry. In situ denitrification capacity rates were also investigated in shallow groundwater at OP underneath a cover crop (mustard) that has been cultivated after spring barley since 2006. The abundance of denitrifier functional genes in groundwater at all study sites on two occasion, May and June 2010, was conducted and reported in this chapter.

6.2 Introduction

Mitigation of NO_3^- contamination includes reduced NO_3^- loading (Silgram et al., 2005; Johnson et al., 2007) and reliance on natural attenuation processes which requires a comprehensive quantitative understanding of denitrification rates, being considered as the major NO_3^- depletion mechanism in groundwater (Boyer et al., 2006; Rivett et al., 2008). Denitrification, a multistep biological process producing NO_2^-, NO, N_2O and N_2 from NO_3^- is carried out mainly by facultative anaerobes which are considered as ubiquitous (Linne von Berg and Bothe, 1992). The obligate intermediate product of denitrification, N_2O, has the global warming potential 300 times of CO_2. There is great interest in the final intermediate product of denitrification, N_2O (Kellogg et al., 2005; Clough et al., 2007; Well et al., 2005; Weymann et al., 2008; Von der Heide et al., 2008) due to its contributions to ozone production and consumption and radiative forcing in the atmosphere (Prather et al., 2001). But the fate, movement and consumption of N_2O in groundwater are poorly understood (Clough et al., 2005, 2007).

The last step of denitrification is critically important because it is a permanent sink for reactive N in the environment. The amount of reactive N that is converted back to N_2 is by far the largest uncertainty of the N cycle on all scales (Galloway et al., 2004). Efficiency of NO_3^- removal via denitrification in groundwater ranges roughly 0% to 100%, which is spatially heterogeneous and depends locally on aquifer hydrology and mineralogy (Korom, 1992; Hiscock et al., 2003); dissolved O_2, microbial expression, energy sources, and redox chemistry (Boyer et al., 2006). The key objectives of this chapter was (a) to quantify the extent of in situ denitrification at three vertical hydrogeochemical gradients and to fractionate its end products (N_2O and N_2) in the light of the indirect N_2O emissions; and to link denitrification products to the existing hydrologic and biogeochemical environments.

6.3 Measurement of Natural NO_3^- Attenuation

6.3.1 Groundwater sampling

Groundwater sampling was carried out on a monthly basis between Feb, 2009 and Jan, 2011 using a bladder pump (Geotech Environmental Equipment, Inc., USA) following USEPA Region I Low Stress Purging and Sampling Procedures (USEPA, 1996) for analysing dissolved gases (N_2O and N_2) and hydrogeochemistry. Triplicate samples were collected through Teflon made water outlet tubing (ID 0.6 cm) at a rate of 100 ml min^{-1} so that withstanding of pressure does not cause any ebullition of dissolved gases. To analyse dissolved N_2, Ar and O_2, samples were collected into a 12 ml exetainer (Labco Wycomb Ltd., UK), after slowly overflowing approximately 10 ml excess water and closed immediately using double septum (butyl rubber + Teflon) stopper. To analyse dissolved N_2O, water samples were collected into 160 ml serum bottles after overflowing of approximately 150 ml water and immediately sealed with butyl rubber septa and aluminium crimp caps (WHEATON, USA). All samples including samples for dissolved gases (dipped under water), were kept in cool boxes, stored at 4° C and analysed within one week. This is an accepted practice/sample treatment in these experimental procedures (Weymann et al., 2009).

6.3.2 Measurement of dissolved gases in groundwater

The exetainer samples for N_2, O_2 and Ar were analysed in a high precision membrane inlet mass spectrometer (MIMS) (Kana et al., 1994). Past studies showed that MIMS can analyse dissolved N_2/Ar with high precision (Kroeger et al., 2006; Singleton et al., 2007). Calibration standards were deionized water equilibrated with air at known temperature and pressure (no salinity was observed). A single point calibration was used in MIMS because the MIMS instrument responded in a linear way to concentration of the gases, and so a single standard was sufficient. The gas concentrations in the standard bath were calculated by the Weiss gas solubility equations (Weiss, 1970). Several readings of the standards were made between sets of 10 samples to test for instrument drift and allow for drift corrections if needed. The sample is pushed past a gas permeable silicon membrane where the vacuum degassed the water. Gases then passed through a liquid nitrogen trap to remove water vapour and CO_2. The CO_2 must be eliminated as CO derived from CO_2 has the same mass of N_2. Sample gases were then drawn into a Pfeiffer Vacuum ^{TM}QMS 200 quadrupole mass spectrometer.

To determine the dissolved N_2O concentrations, samples were degassed using high purity *He* (BOC, Linde Group, Germany) (*He*: water 1:3; v/v) following the headspace equilibration technique. After equilibration, headspace gas sample was collected in a 12 ml exetainer (Labco Wycombe Ltd., UK) with an additional injection of 12 ml *He* using a PVC syringe. The N_2O was analysed in auto sampler gas chromatograph (CP-3800, Varian, Inc. USA) equipped with an electron capture detector (ECD) using Ar as a carrier gas. The N_2O concentrations in water samples were estimated using the Henry's Law. The partial pressures of N_2O in equilibrated headspace and water were calculated using its solubility (Weiss, 1970) at the recharge temperature as measured at the interface between the unsaturated zone and groundwater surface.

Denitrified N_2, presented as excess N_2 (Heaton and Vogel, 1981), was estimated using

$$X_{N_2Excess} = X_{N_2T} - X_{N_2WEA} - X_{N_2ExcessAir} \qquad \text{(Eqn. 6.1)}$$

where $X_{N_2Excess}$ = excess N_2 concentration from denitrification, X_{N_2T} = molar concentration of total dissolved N_2, X_{N_2WEA} = molar concentration of N_2 in water equilibrium with atmosphere and $X_{N_2ExcessAir}$ = molar concentration of N_2 in entrapped air

bubble. The X_{N_2WEA} data were estimated from N_2 solubility data (Weiss, 1970) based on groundwater recharge temperature as measured at the interface between unsaturated zone and the aquifer (Heaton and Vogel, 1981). For a given recharge temperature, entrapped air is reflected by noble gas concentrations which is subject to complete or partial dissolution (Holocher et al., 2002). If entrapped air N_2 results from complete dissolution of air bubbles, the composition of entrapped air will be identical to the composition of atmospheric air. The entrapped air N_2 concentration can be calculated from the concentration of only one noble gas, e.g. Ar (Heaton and Vogel, 1981). Therefore, the entrapped air N_2 concentration can be calculated using:

$$X_{N_2 Excess\ Air} = (X_{Ar\,T} - X_{Ar\,WEA}) * \frac{X_{N_2 ATM}}{X_{Ar\,ATM}} \qquad \text{(Eqn. 6.2)}$$

where $X_{Ar\,T}$ = Total dissolved Ar concentration, $X_{Ar\,WEA}$ = Ar concentration in water equilibrium with atmosphere, estimated from the solubility data of Ar (Weiss, 1970) at this specific recharge temperature; $X_{N_2 ATM}$ = atmospheric mole fractions of N_2 and $X_{Ar\,ATM}$ = atmospheric mole fractions Ar. Conversely, if entrapped air results from the incomplete dissolution of entrapped air bubbles which depends on the hydrostatic pressure on the entrapped air bubbles during infiltration (Heaton and Vogel, 1981; Holocher et al., 2002), then the N_2:Ar ratio of entrapped air is lower than the atmospheric composition due to fractionation (Holocher et al., 2002) because incomplete gas dissolution enriches the more soluble gas in the infiltrating water. This effect lowers the true N_2:Ar ratio and thus results in an underestimate of calculated N_2 from denitrification. Similarly, an actual recharge temperature lower than the assumed value would also lead to an underestimated N_2 from denitrification (Beller et al., 2004). But the minimum value of the N_2:Ar ratio of entrapped air is equal to the N_2:Ar ratio in water at atmospheric equilibrium (Aeschbach-Hertig et al., 2002). The minimum estimates of [N_2 Excess Air] are thus given by:

$$X_{N_2 Excess\ Air} = (X_{Ar\,T} - X_{Ar\,WEA}) * \frac{X_{N_2 WEA}}{X_{Ar WEA}} \qquad \text{(Eqn. 6.3)}$$

Therefore, excess N_2 was estimated based on the average values of equations 6.2 and 6.3 (Weymann et al., 2008).

6.3.3 Estimation of initial NO_3^--N concentration, N_2O emission factor, N_2O mole fraction and reaction progress (RP)

From the assumption that NO_3^--N concentration along groundwater flow path between the aquifer surface and a given sampling spot originates from denitrification and results in quantitative accumulation of gaseous denitrification products (N_2O and N_2), it follows that initial NO_3^--N (N ini) concentration can be calculated from the sum of residual substrate and accumulated products (Böhlke, 2002). Therefore, initial NO_3^--N concentration can be estimated by:

$$NO_3^- - N_{ini} = N_2O\text{-}N + N_{2\ Excess}\text{-}N + NO_3^-\text{-}N \qquad \text{(Eqn. 6.4)}$$

Reaction progress (RP) was estimated as the ratio between products and starting material of a process and can be used to characterise the extent of NO_3^--N elimination by denitrification (Böhlke et al., 2007; Weymann et al., 2008). Thus the RP was calculated using the following equation:

$$RP = \frac{N_2O - N + N_{2\,Excess} - N}{NO_3^- - N_{ini}} \qquad \text{(Eqn. 6.5)}$$

The N_2O emission factors (EFg) for indirect N_2O emission from groundwater was estimated after by Weymann et al. (2008):

$$EFg(1) = \frac{N_2O - N}{NO_3 - - N_{ini}} \qquad \text{(Eqn. 6.6)}$$

The emission factor, used by many researchers (Reay et al., 2003; Sawamoto et al., 2003), assuming that NO_3^--N and N_2O do not transform while transported into and through aquifer as proposed by IPCC (1997), can be calculated by using:

$$EFg(2) = \frac{N_2O - N}{NO_3 - - N} \qquad \text{(Eqn. 6.7)}$$

The N_2O mole fraction was estimated using:

Mole fraction = N_2O-N/TDN (Eqn. 6.8)

where TDN is the summation of denitrification end products (N_2O-N+excess N_2-N).

6.3.4 Quantifying denitrifier functional genes in groundwater

Denitrifier functional genes (gene copy concentration, GCC) were quantified in the Laboratory of Microbial Ecology, NUI Galway. Five litres groundwater samples were collected from each well in two subsequent months (May and June 2009) to analyse the abundances of denitrifier harbouring genes e.g. *nir* (*nirK and nirS*) and *nosZ* genes in the National University of Ireland Galway (Barrett et al., 2010). DNA was concentrated by vacuum filtration on 0.2 µm filter paper. Functional gene abundances were quantified using real-time PCR assays targeting the NO_3^- reductase (*nir*) and nitrous oxide reductase (*nos*) genes. The abundance of the denitrifier genes (gene copy concentrations, GCC) were related here with the groundwater hydrogeochemistry and denitrification end products (N_2O and excess N_2).

6.3.5 Statistical analysis

Analysis was performed using the Mixed Procedure (SAS, 2009). As most of the variables showed an approximately lognormal distribution, log transformations were used with appropriate re-scaling so that residual checks indicated that the assumptions of the analyses were not violated. Pre-specified hypotheses of influential variables were tested by regression modelling for both N_2O and excess N_2. Sequential addition of the variables to the model was performed where the size of the F statistic gives an indication of their relative contribution to the full model. Structural factors like depth and sampling dates were tested. Covariance models were included to account for correlations in the data (e.g. across sampling date). For the concentrations of denitrification end products, N_2O mole fractions and emission factors, effects of location and depth were examined along with their interactions. In case significant differences were found, Tukey Kramer HSD all pairs multiple comparison test were used to distinguish differences between individual site and depth.

6.4 Results of quantifying in situ N_2O and excess N_2

6.4.1 Spatial and temporal variability in N_2O and excess N_2 concentrations

The N_2O-N concentrations in groundwater differed significantly between sites ($p<0.001$), but were similar among depths at each site ($p>0.05$) except at JC where it showed significantly higher ($p<0.05$) N_2O in subsoil than at interface and bedrock (Figure 6.1). Regardless of depth, mean N_2O concentrations over the two years (Feb, 2009- Jan, 2011) were 0.024, 0.011, 0.038 and 0.049 mg N L^{-1}, respectively at JC, SH, OP and DG. There was no significant difference in N_2O concentrations between 2009 and 2010 ($p>0.05$) at the study sites unlike SH where it was higher ($p<0.05$) in 2009 than that in 2010. Temporal variations in N_2O concentrations were remarkable across sites and depths with highest concentrations during February to April and lowest during August to October (Figure 6.2). In connection with these fluctuations over time, N_2O measurement was carried out for three subsequent days with the coincidence of heavy rainfall during November 2009 in few wells at JC. Rainfall increased GWT gradually by 50 cm during the 3-day and N_2O concentrations increased concurrently with the rise in GWT. A comparatively higher spatial variability (than temporal) of N_2O concentrations were observed at all sites and depths (Appendix 11). There were few wells at JC and most of the wells at SH where very low (0.01-0.02 mg N L^{-1}) N_2O concentrations were measured, whereas it was as high as 0.14 mg N L^{-1} in some other wells. The coefficients of variation ranged from 87-126, 91-149, 56-81 and 82%, respectively, at JC, SH, OP and DG, irrespective of depth.

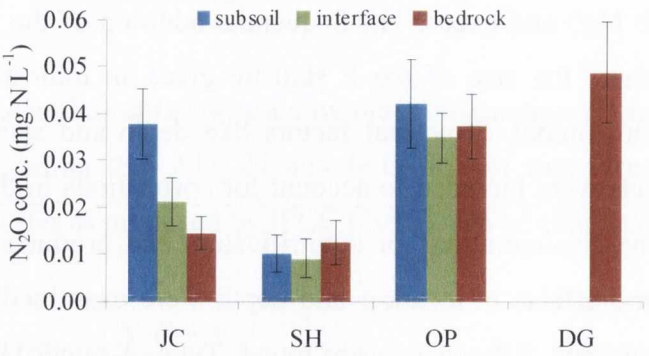


Figure 6.1 Mean N_2O concentrations in three different depths of groundwater; (S) subsoil, (I) interface and (B) bedrock at four sites; (a) JC, (b) SH, (c) OP and (d) DG during the sampling period of Feb, 2009 to Jan, 2011

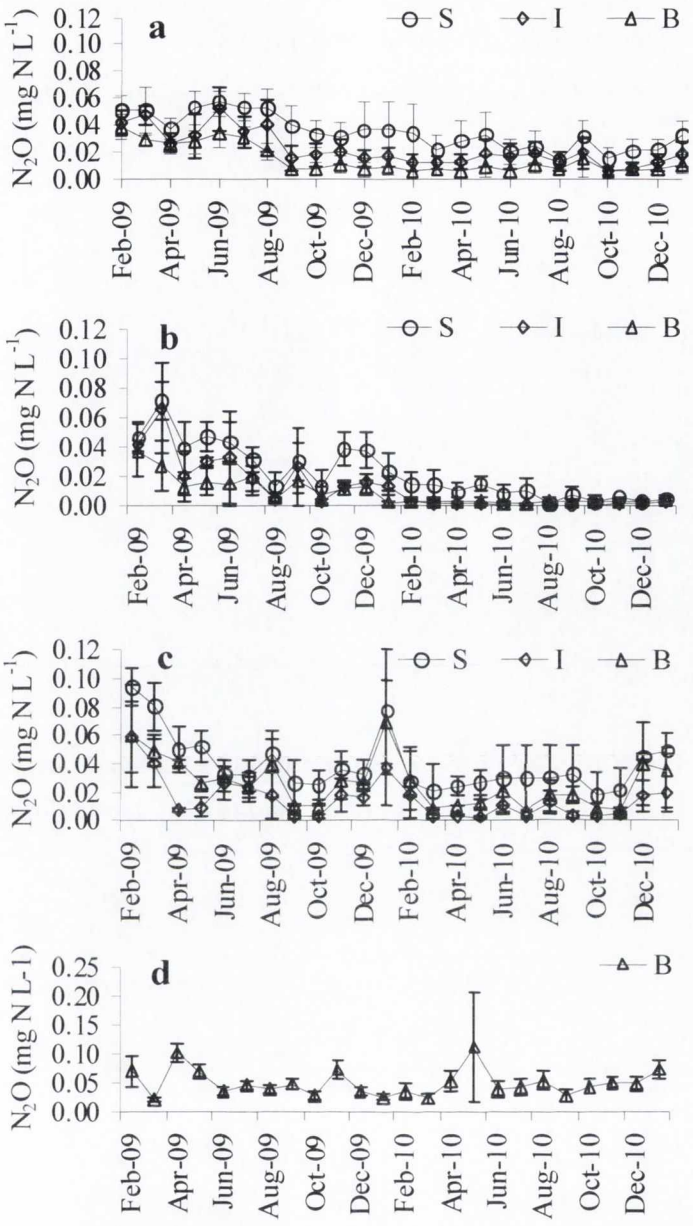


Figure 6.2Mean (±SE) N_2O concentrations in three different depths of groundwater; (S) subsoil, (I) interface and (B) bedrock at four sites; (a) JC, (b) SH, (c) OP and (d) DG during the sampling period Feb, 2009 to Jan, 2011

Mean excess N_2 (denitrified N_2) concentrations in groundwater between depths ranged from 1.72 - 2.62, 2.18 - 2.56, 0.69 - 1.19 and 0.46 mg N L^{-1} with corresponding mean values of 2.28, 2.33, 0.90 and 0.45 mg N L^{-1}, respectively, at JC, SH, OP and DG (Figure 6.3). Excess N_2 was significantly different between sites ($p<0.001$) but was similar among depths, except at OP where it was significantly higher in bedrock than subsoil and interface ($p<0.001$). Excess N_2 concentrations were moderately variable over time with highest

concentrations observed in July to October and lowest December to February regardless of site or depth (Figure 6.4). Excess N_2 had a remarkably high spatial variability showing the coefficients of variation 55-82, 45-65, 36-69 and 74%, respectively, at JC, SH, OP and DG. There were few wells at JC and SH with comparatively higher excess N_2 concentrations (3.5-8.69 mg N L^{-1}) than other wells (Appendix 11).

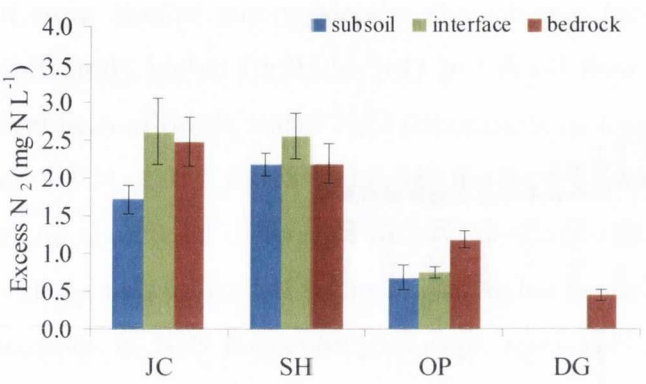


Figure 6.3 Mean excess N_2 concentrations in three different depths of groundwater; (S) subsoil, (I) interface and (B) bedrock at four sites; (a) JC, (b) SH, (c) OP and (d) DG during the sampling period Feb, 2009 to Jan, 2011

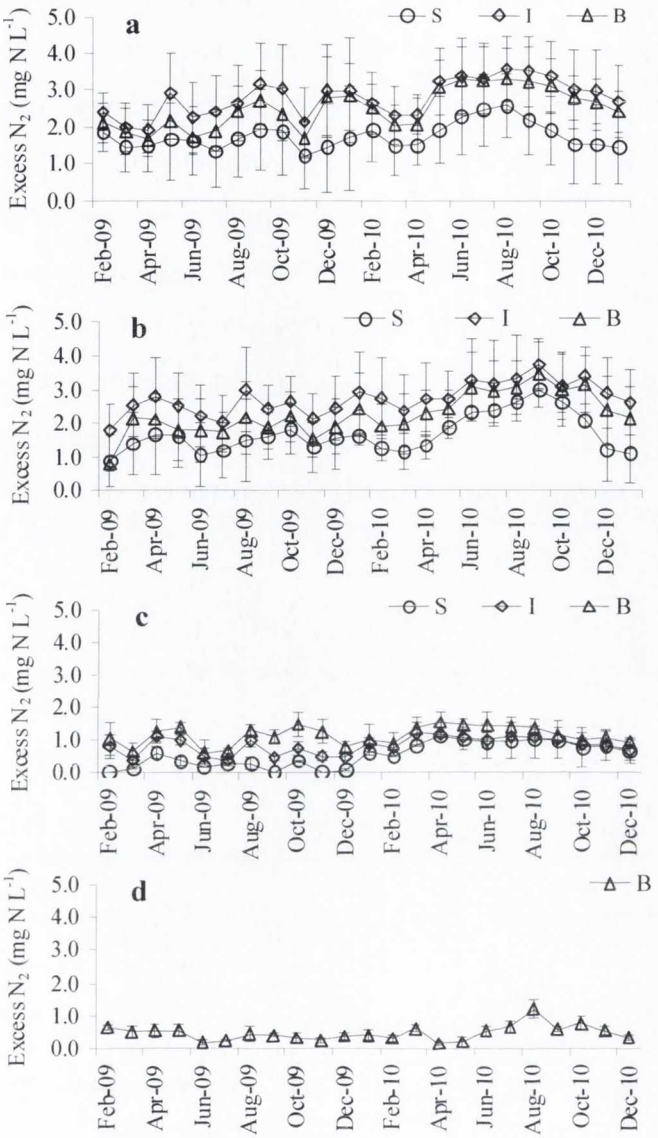


Figure 6.4 Mean (±SE) excess N_2 concentrations in three different depths of groundwater; (S) subsoil, (I) interface and (B) bedrock at four sites; (a) JC, (b) SH, (c) OP and (d) DG during the sampling period Feb, 2009 to Jan, 2011

6.4.2 Groundwater as a source of atmospheric N_2O: mole fractions and emission factors

Groundwater N_2O was observed at 2-3 orders of magnitude higher than the atmospheric concentration at all sites and depths. Mean N_2O mole fractions (N_2O/N_2O+N_2) were 0.02, 0.01, 0.09 and 0.19, respectively, at JC, SH, OP and DG sites. N_2O mole fraction in subsoil and in bedrock significantly differed between sites ($p<0.001$), but at the interface it was similar ($p>0.05$). At JC and OP, it was significantly higher in subsoil than at the interface

and bedrock ($p<0.001$), but at SH it was similar among depths. N_2O mole fraction significantly decreased and excess N_2 increased (Figure 6.5), suggesting that N_2O further reduced to N_2. N_2O emission factors (EF) according to the IPCC (2006) methodology was considerably higher than the IPCC default at JC and SH sites but was similar at OP and DG sites (Table 6.1). Mean EF according to the IPCC methodology was 0.0257, 0.0286, 0.0044 and 0.0033, respectively, at JC, SH, OP and DG. When another method proposed by Weymann et al. (2008) was used, it was 0.0044, 0.0043, 0.0035 and 0.0032, respectively, at JC, SH, OP and DG. Emission factor varied significantly between sites at interface and bedrock ($p<0.05$), but in subsoil the difference was absent ($p>0.05$). There were no significant differences among depths at individual site ($p>0.05$).

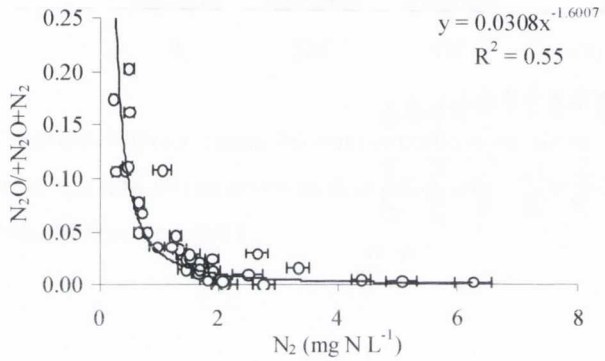


Figure 6.5 Plots, N_2O mole fractions vs. excess N_2 using mean data of the whole study period (n=36)

6.4.3 Initial NO_3^- loadings and NO_3^- removal by denitrification

Mean initial NO_3^--N concentration (NO_3^- that delivered or leached out to groundwater from soil i.e., N_2O+excess N_2+NO_3^--N) was 6.0, 3.0, 12.0 and 15.0 mg N L^{-1}, respectively at JC, SH, OP and DG (Figure 6.6). Mean initial NO_3^- loadings varied significantly between sites ($p<0.001$), but at each site, it was similar among depths except at OP site where it increased significantly with depths ($p<0.001$). The JC and SH showed significantly lower initial NO_3^--N than OP and DG, but no significant differences were observed between JC and SH and between OP and DG. Mean TDN (N_2O+excess N_2) were 2.30, 2.34, 0.93 and 0.51 mg N L^{-1}. TDN differed significantly between sites ($p<0.001$) except between JC and

SH. Interestingly, TDN did not differ significantly between depths at each site ($p>0.05$) except OP where it was significantly higher in bedrock than subsoil and interface ($p<0.01$).

Mean losses of initial NO_3^--N via denitrification, expressed by the reaction progress (TDN over initial NO_3^--N), were 0.46, 0.77, 0.08 and 0.04, respectively, at JC, SH, OP and DG (Figure 6.7). There was a significant difference between sites ($p<0.001$). No significant differences were observed among depths at each site unlike JC where it was significantly lower in subsoil than at interface ($p<0.001$) and bedrock ($p<0.001$). Singular regression analysis showed strong negative correlation between ambient NO_3^--N values and mean RP (Figure 6.8), TDN (Figure 6.9) and excess N_2 (Figure 6.10) suggesting that initial nitrate concentrations decreased due mainly to the denitrification processes.

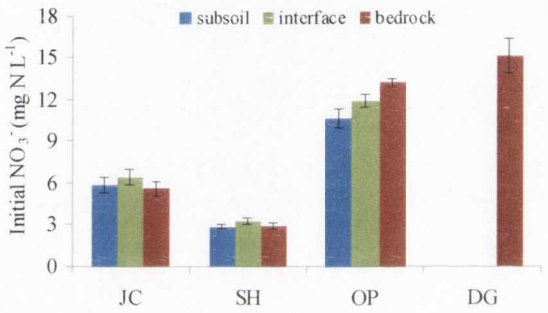


Figure 6.6Mean initial nitrate loadings to groundwater in three different depths of groundwater; (S) subsoil, (I) interface and (B) bedrock at four sites; (a) JC, (b) SH, (c) OP and (d) DG during the sampling period Feb, 2009 to Jan, 2011

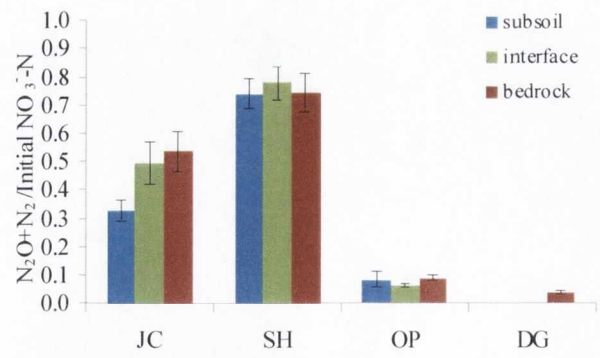


Figure 6.7Mean RP (N_2O+excess N_2/N_2O+excess N_2+NO_3^-) in three different depths of groundwater; (S) subsoil, (I) interface and (B) bedrock at four sites; (a) JC, (b) SH, (c) OP and (d) DG during the sampling period of Feb, 2009 to Jan, 2011

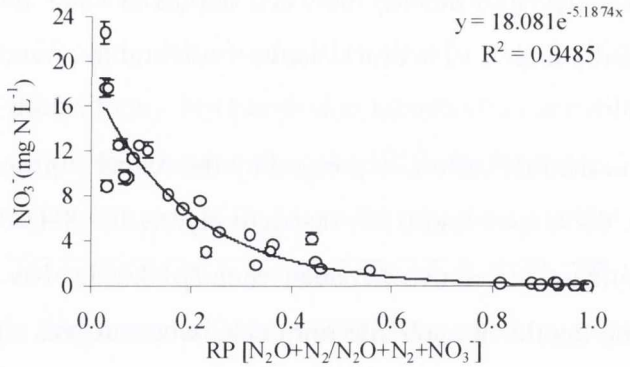


Figure 6.8Plots, groundwater NO_3^--N concentrations vs. RP {(N_2O-N + excess N_2-N)/Initial NO_3^--N)} using the mean data of the whole study period (n=36)

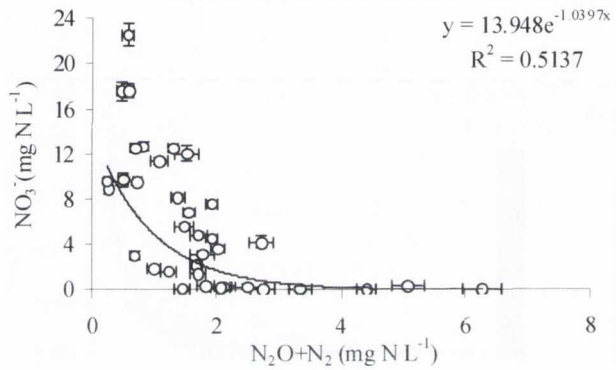


Figure 6.9Plots, nitrate concentrations vs. TDN (N_2O+excess N_2) using mean data of the whole study period (n=36)

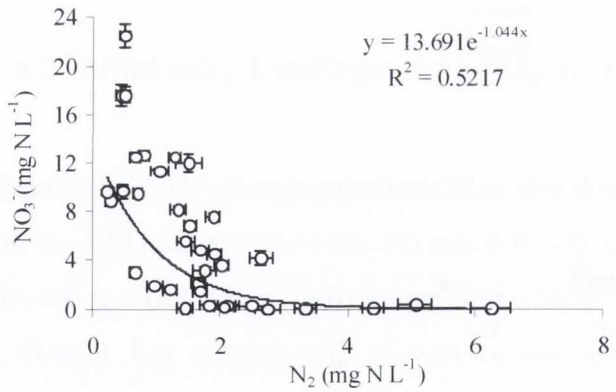


Figure 6.10Plots, nitrate concentrations vs. excess N_2 using mean data of the whole study period (n=36)

Table 6.1Groundwater TDN (N_2O+excess N_2), N_2O mole fraction and emission factors across sites and depths (mean ± SE; n=24)

Location/ Geological position of screen	TDN* (mg N L^{-1})	N_2O mole fraction (N_2O/N_2O + excess N_2)	N_2O emission factor (N_2O-N/NO_3^--N)	N_2O emission factor* (N_2O-N/initial NO_3^--)
Johnstown castle				
Subsoil	1.76±0.19	0.03±0.01	0.0124±0.0036	0.0069±0.0015
Bedrock-interface	2.64±0.43	0.01±0.00	0.0540±0.0177	0.0031±0.0007
Bedrock	2.50±0.33	0.01±0.00	0.0108±0.0097	0.0026±0.0014
Solohead				
Subsoil	2.19±0.17	0.01±0.00	0.0577±0.0183	0.0033±0.0013
Bedrock-interface	2.57±0.30	0.01±0.01	0.0162±0.0096	0.0032±0.0014
Bedrock	2.21±0.27	0.01±0.01	0.0119±0.0066	0.0060±0.0029
Oak Park				
Subsoil	0.74±017	0.16±0.06	0.0063±0.0020	0.0047±0.0012
Bedrock-interface	0.79±0.07	0.06±0.01	0.0042±0.0005	0.0033±0.0004
Bedrock	1.23±0.11	0.05±0.03	0.0031±0.0006	0.0029±0.0005
Dairy Gold				
Bedrock	0.51±0.08	0.19±0.06	0.0033±0.0006	0.0032±0.0006

*TDN is total denitrification (N_2O + excess N_2); initial NO_3^--N is N_2O + excess N_2 + NO_3^--N

6.4.4 Distributions of NO_3^- and N_2O+excess N_2 along the transect of agricultural catchment

Nitrate and N_2O+excess N_2 concentrations showed a decreasing trend along transect from groundwater divide towards the discharge at JC (Figure 6.11). Groundwater divide was shown as the distance zero (0) along the x axis, located at the highest elevation (m AOD), and the distances and elevations of other wells along transect were shown from the divide to the discharge (bottom most well). Distributions of NO_3^--N and excess N_2-N showed a very complex and heterogeneous pattern at JC. In well JC2B and JC2C, even they are nearly top of the transect, showed highest N_2O+excess N_2-N and no NO_3^--N. In these wells DOC were higher than other wells together with a favourable hydrogeochemical environments e.g., low DO and Eh (Appendix 9) for denitrification. In wells JC3A, JC3B and JC3C, even they are in the middle of transect, NO_3^--N concentrations were higher than other wells. This can be related to the ambient hydrogeochemical conditions, being with lower DOC and higher DO and Eh at JC3A, JC3B and JC3C (Appendix 9). However, the remaining wells showed a pattern of decreasing NO_3^--N distribution with concomitant increasing in N_2O+excess N_2-N concentrations, being with lowest in the wells at the down most gradient (e.g., JC32, JC33 and JC34). At SH site, the distribution pattern along the

transect was very distinctive with the lowest N_2O +excess N_2 in the top gradient wells and highest in the down gradient wells (Figure 6.12). Similarly, higher N_2O +excess N_2 were observed in the down gradient wells at OP site (Figure 6.13), even though nitrate were also higher in the down gradient wells. This can be attributed to the addition of NO_3^- in the field into groundwater as the wells were at the end of the field and soils and subsoils were free drained under arable systems. Arable systems facilitate more nitrification and thus higher NO_3^- accumulation into groundwater in well drained conditions than the grassland. Denitrification at DG was very negligible, therefore no distinct pattern of N_2O+excess N_2 was observed (Figure 6.14).

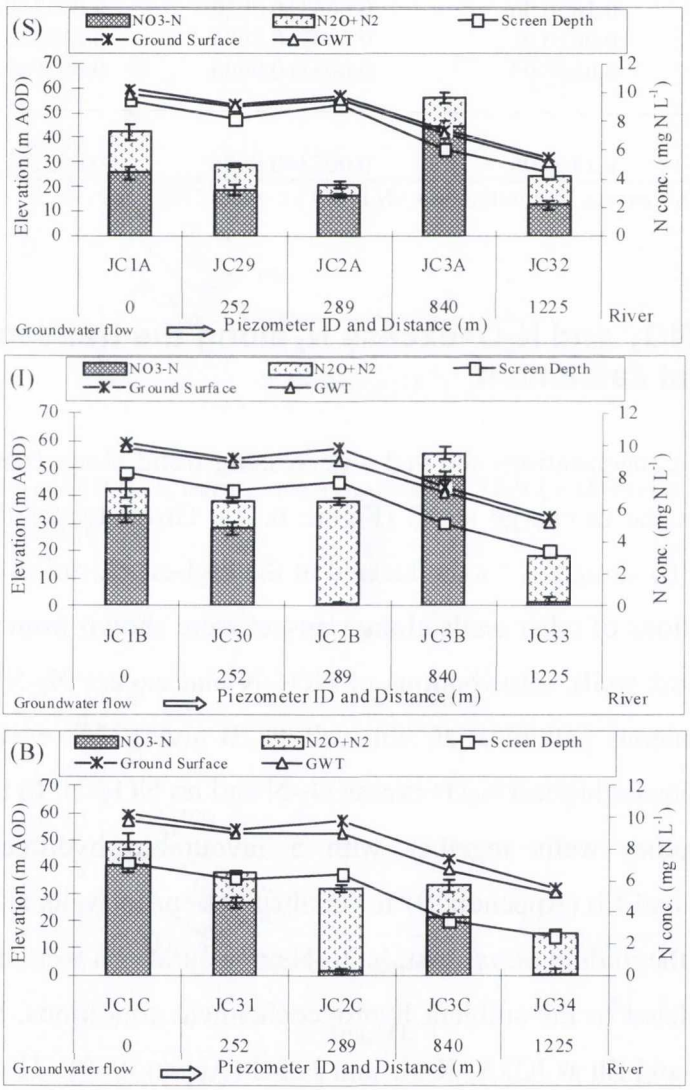


Figure 6.11Nitrate and N_2O+excess N_2 distributions along the transect of groundwater flow paths at JC

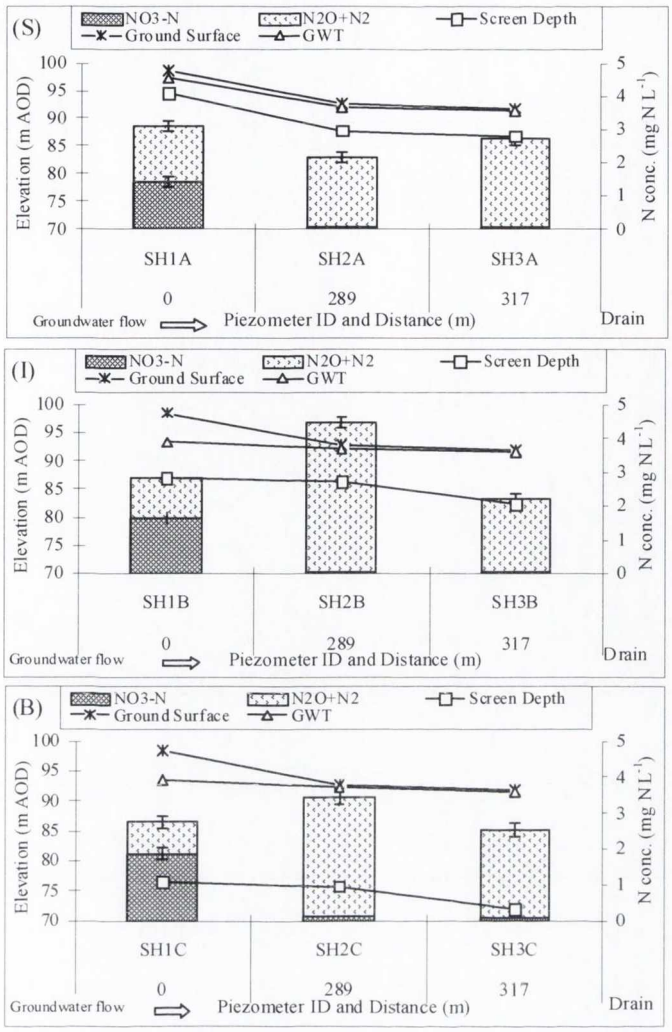


Figure 6.12Nitrate and N_2O+excess N_2 distributions along the transect of groundwater flow paths at SH

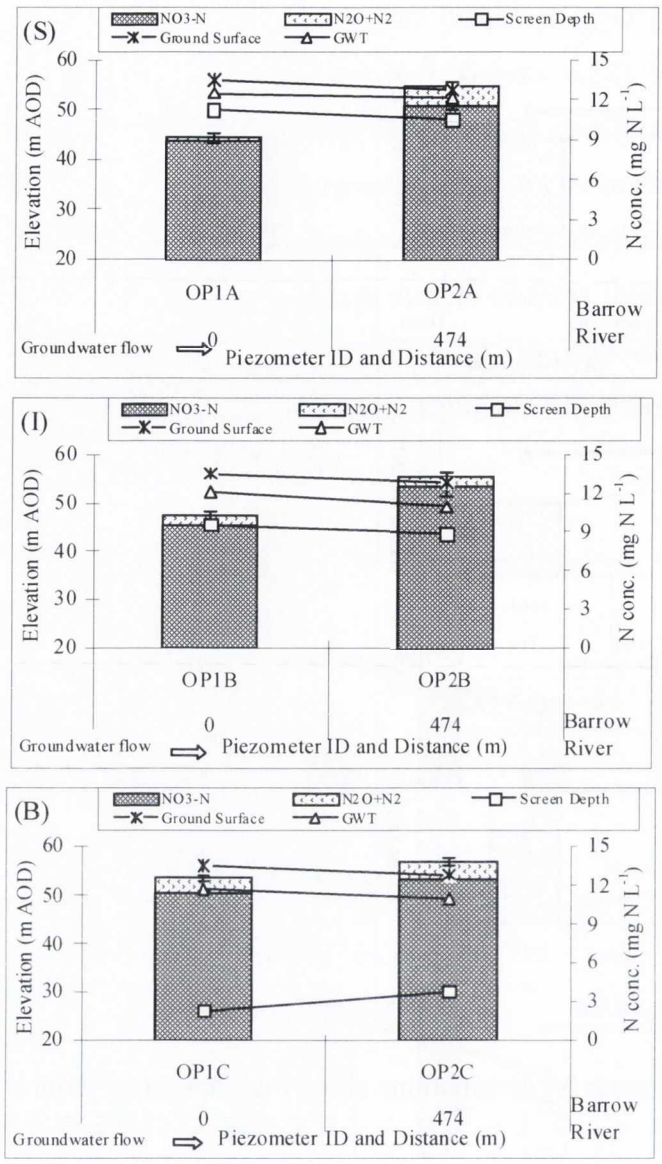


Figure 6.13Nitrate and N_2O+excess N_2 along the transect of groundwater flow paths at OP

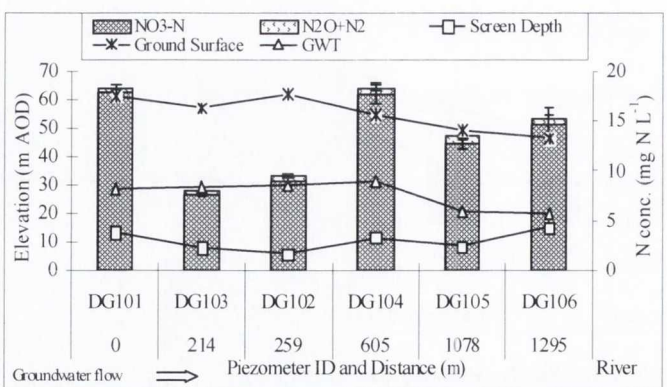


Figure 6.14Nitrate and N_2O+excess N_2 along the transect of groundwater flow paths at DG

6.4.5 Abundances in denitrifier functional genes in groundwater

Denitrifier functional genes (Gene Copy Concentration-GCC) were detected across all sites and depths but their abundance was similar ($p>0.05$) at all sites and depths (Figure 6.15). The mean bacterial abundances were similar at the interface (6.7×10^4 genes L^{-1}), subsoil (2.9×10^4 genes L^{-1}) and bedrock (2.6×10^4 genes L^{-1}). The most abundant denitrifying functional genes were *nirS* (nitrite reductase that contains heme c and heme d1; cd1-Nir), ranged 1.4×10^4 genes L^{-1} in subsoil to 1.2×10^4 genes L^{-1} in bedrock followed by *nosZ* (nitrous oxide reductase), varied 1.1×10^3 genes L^{-1} in subsoil to 1.9×10^3 genes L^{-1} at interface. The *nirK* (NO_3^- reductase that contains copper; Cu-Nir) were similar at interface (3.8×10^1 genes L^{-1}), subsoil (6.2×10^1 genes L^{-1}) and bedrock (1.5×10^2 genes L^{-1}). The denitrifier genes to bacteria ratios {(*nirK*+*nirS*+*nosZ*)/bacteria} were similar ($p>0.05$) across sites and among depths in each site, ranging among depths from 0.38-0.84, 0.55-0.97, 0.06-0.72 and 0.58 respectively at JC, SH, OP and DG.

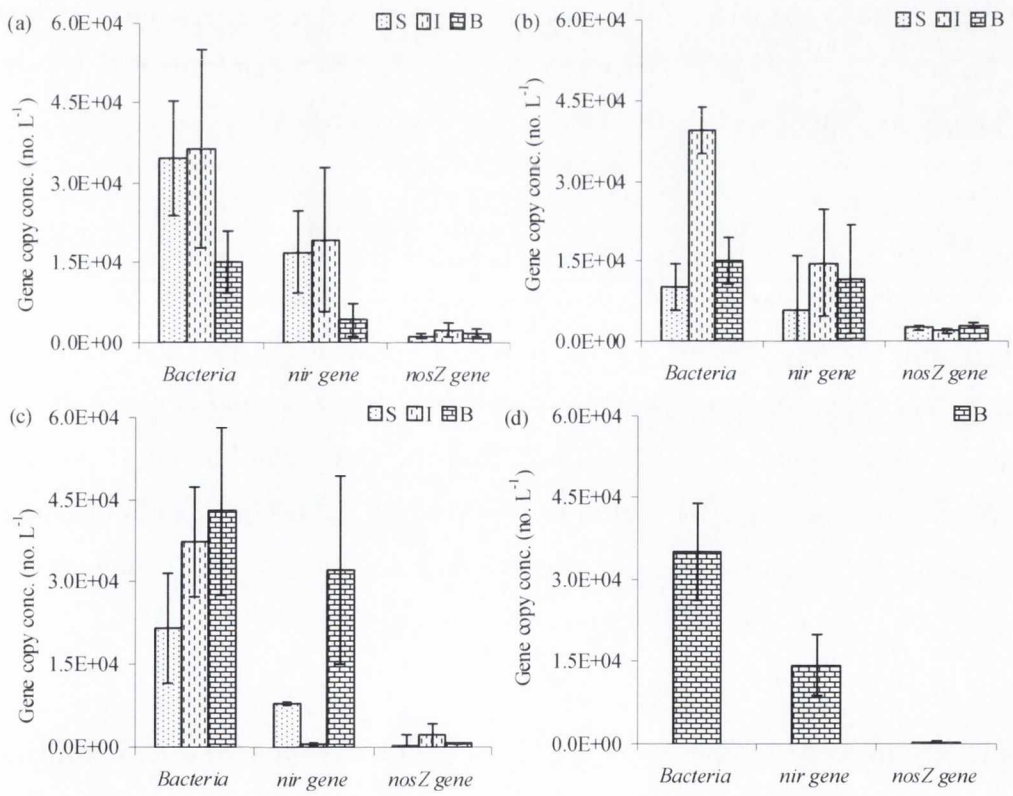


Figure 6.15Relative abundances of denitrifier functional genes in three different zones of groundwater; (S) subsoil, (I) interface and (B) bedrock at (a) JC, (b) SH, (c) OP and (d) DG

6.4.6 Environmental drivers of groundwater denitrification

6.4.6.1 Relationships between N_2O and the ambient hydrogeochemical conditions

Pearson's correlation coefficient analysis revealed that groundwater N_2O concentrations increased with decreasing ratio of depth below water table (bwt) to depth bgl, K_{sat} values and T°C (Table 6.2). Groundwater Eh and DO showed strong positive linear relationships with N_2O (Table 6.2). Other important covariates of N_2O emissions in groundwater were DOC, NO_3^--N and SO_4^{2-} concentrations which showed significant positive relationships with N_2O (Table 6.2). Groundwater pH, NH_4^+-N, excess N_2 and RP increased with decreasing N_2O concentrations (Table 6.2). Groundwater bacterial, *nir* (NO_3^- reductase), *nir/(nir+nosZ)* and *nosZ* (nitrous oxide reductase) genes showed comparatively weak relation with N_2O showing r values of 0.110, 0.151, 0.211 and -0.183 with corresponding p values of 0.143, 0.109, 0.068 and 0.086.

Multiple linear regressions following the stepwise method based on the changes in F-values revealed a mixed model where logEh, logNO_3^--N, and logDOC showed positive relationships with predicted N_2O concentrations given below:

$$\log N_2O = -1.6001 + 0.4809\ \log Eh + 0.5308\ \log NO_3^-\text{-}N + 0.1876\ \log DOC \quad (R^2=0.58;\ p=0.001;\ n=806) \qquad \text{(Eqn. 6.9)}$$

where N_2O concentration, NO_3^--N and DOC are in mg L^{-1} and Eh is in mV. The model sequentially included the variables with their relative contributions (F values of 78.22, 20.76, 7.69, respectively for logEh, logNO_3^--N and logDOC). In addition, sampling dates have significant contributions to the model giving F value of 18.22 (highest conc. in Feb-Mar) that affect the intercept of the model over time.

6.4.6.2 Relationships between excess N_2 and hydrogeochemical conditions

Excess N_2 in groundwater showed significant positive correlations with the ratio of depth bwt to depth bgl (Table 6.2). Excess N_2 showed an inverse relationship with groundwater $K_{sat,}$ but linear positive relationship with T°C (Table 6.2). Most important factors of excess

N_2 production were DO and Eh as revealed from the negative relationships with excess N_2 and DO ($p<0.001$) as plotted in Figure 6.16 and Eh ($p<0.001$) as plotted in Figure 6.17. Groundwater NH_4^+-N showed significant positive correlation with excess N_2 (Table 6.2). Surprisingly, DOC showed negative correlation with excess N_2 while pH and SO_4^{2-} concentrations showed positive correlation (Table 6.2). Conversely, increased excess N_2 significantly and positively correlated with Fe^{2+}, Mn^{2+} but negatively correlated with S^{2-} concentrations (Table 6.2). NO_3^--N, NO_2^--N and N_2O concentrations showed significant negative correlation with excess N_2 (Table 6.2). A strong negative correlation between excess N_2 and reaction progress ($N_2O+N_2/N_2O+N_2+NO_3^-$) indicated that excess N_2 in groundwater was derived from the nitrate that leached out from below the rooting zone, via denitrification (Figure 6.18). Groundwater denitrifiers' abundances e.g., *nir/(nir+nosZ)* showed negative ($r=-0.232$; $p=0.057$) and *nosZ* showed positive correlation ($r=0.398$; $p=0.012$) with excess N_2. Multiple linear regressions for excess N_2 estimated the best fit model which showed negative relationships between excess N_2 and logDO, logEh and positive relationships with temperature as shown below:

log excess N_2 = 12.5198 - 0.5795 logDO - 1.2894 log Eh + 0.1144 temperature ($R^2=0.66$; $p=0.001$; $n=792$, not all data were used in the model) (Eqn. 6.10)

where excess N_2, and DO are in mg L^{-1} and Eh is in mV and log DO, log Eh and temperature respectively showed the F values of 128.63, 16.41 and 7.38. In addition, sampling dates have significant role on excess N_2 concentrations (F value 4.71) contributing substantial changes in the intercept of the model (lowest Nov-Dec).

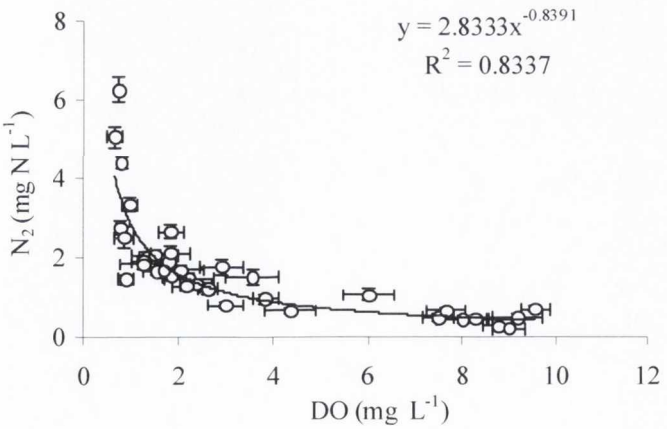


Figure 6.16Plots, Excess N_2 vs. DO in groundwater (mean ±SE; n=36)

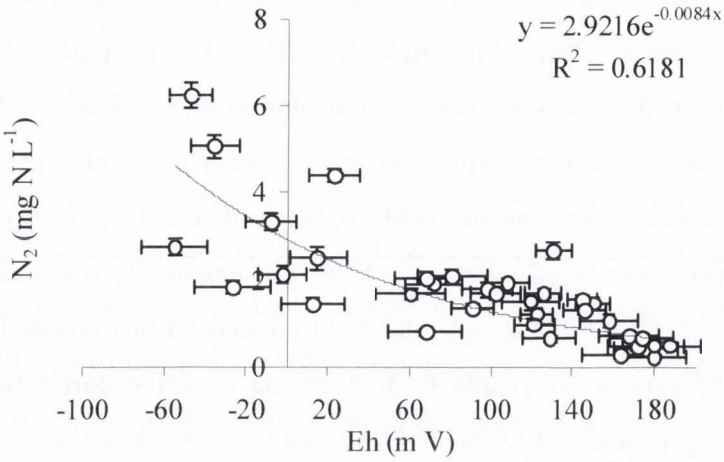


Figure 6.17Plots, Excess N_2 vs. redox potential (Eh) in groundwater (mean ±SE; n = 36)

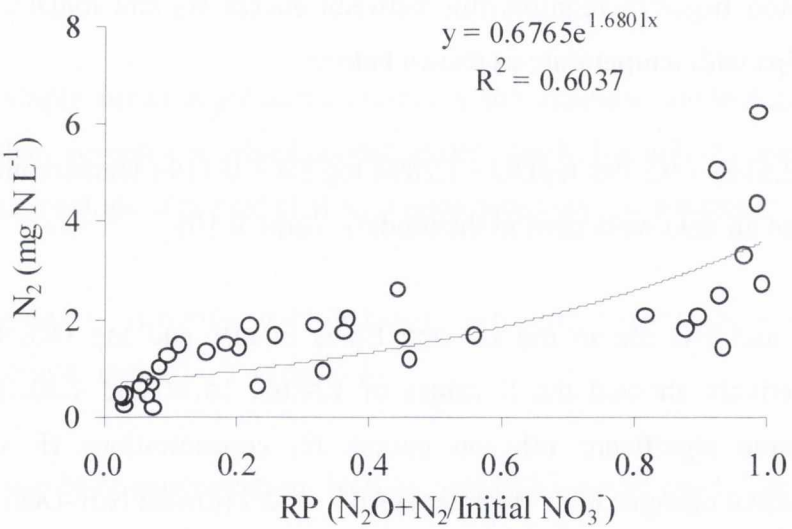


Figure 6.18 Plots, Excess N_2 vs. reaction progress (RP) in groundwater (n = 36)

Table 6.2 Pearson's correlation co-efficient (r) between groundwater hydrogeochemical properties (n=36)

	N_2O	Excess N_2	NO_3^--N	NO_2^-	NH_4^+	GWT	K_{sat}	DO	Eh	Temp	pH	DOC	SO_4^{2--}	S^{2-}	Fe^{2+}	Mn^{2-}
N_2O																
Excess N_2	-0.54**															
NO_3^--N	0.69**	-0.76**														
NO_2^-	0.03 ns	0.09 ns	0.30*													
NH_4^+	-0.31*	0.29*	-0.20 ns	0.51**												
GWT	0.50**	-0.70**	0.66**	0.05 ns	-0.14 ns											
K_{sat}	0.32*	-0.48**	0.47**	-0.02 ns	-0.20 ns	0.63**										
DO	0.60**	-0.90**	0.80**	-0.06 ns	-0.29*	0.65**	0.44**									
Eh	-0.73**	-0.81**	0.79**	-0.06 ns	-0.41*	0.64**	0.52**	0.87**								
Temp	0.37*	0.35*	0.26*	-0.23*	-0.23*	0.55**	0.56**	0.38*	0.40*							
pH	-0.40*	0.01 ns	0.02 ns	0.20 ns	0.03 ns	-0.24*	0.15 ns	0.01 ns	-0.20 ns	-0.24*						
DOC	0.68**	-0.11ns	-0.32*	-0.20 ns	0.25*	0.00 ns	-0.17 ns	-0.06 ns	-0.18 ns	0.22*	-0.31*					
SO_4^{2--}	0.34*	-0.07 ns	0.36*	0.20 ns	0.14 ns	0.25*	0.39*	0.20 ns	0.17 ns	0.09 ns	0.13 ns	0.13 ns				
S^{2-}	0.07 ns	0.12 ns	-0.03 ns	0.13 ns	0.04 ns	-0.30*	-0.38*	-0.03 ns	-0.01 ns	-0.36*	-0.04 ns	-0.22*	-0.36*			
Fe^{2+}	-0.27*	0.66**	-0.52**	-0.32*	0.06 ns	-0.11 ns	0.03 ns	-0.32*	-0.42*	0.22*	-0.01 ns	0.46**	0.06 ns	-0.20 ns		
Mn^{2-}	-0.60**	0.75**	-0.76**	-0.01 ns	0.37*	-0.55**	-0.43*	-0.73**	-0.68**	-0.26*	-0.17 ns	0.16 ns	-0.21	0.01 ns	0.36*	

ns nonsignificant; *significant at 5% level; ** significant at 1% level

6.4.7 Interpretation of the natural NO_3^- attenuation results

6.4.7.1 Groundwater denitrification: Indirect N_2O emissions

The potential role of groundwater ecosystems on global and regional N_2O budgets in NO_3^- contaminated areas are in agreement with other studies (Reay et al., 2003; Well and Butterbach-Bahl, 2010). In the current study, sites a wide range of variations in N_2O concentrations were observed, indicating individual site effects on such emissions. Higher N_2O concentrations at OP and DG could be due the existing environmental conditions which were unfavourable for transformations of N_2O further to N_2, due to comparatively thick USZ (normalized by the ratio of depth bwt to depth bgl) with high permeability and low anaerobicity (high DO and Eh), resulting in high NO_3^--N concentrations, which are known to be prohibitive of N_2O reduction to N_2 (Simek and Cooper, 2002). In these study sites, N_2O production was observed in a wide range of DO suggesting that N_2O production in comparatively aerobic conditions in groundwater might take place in some microsites. Moreover, even though aerobic denitrification in soils (~80% air saturation) was reported by Carter et al. (1995), in groundwater, denitrification actually seems more likely under locally anaerobic conditions within microsites in particulate organic matter (Hammersley and Howes, 2002), heterogeneous organic-rich patches of sediments (Jacinthe et al., 1998) or biofilms (Seiler and Vomberg, 2005). The denitrification process may stop with the formation of NO_x where oxygen levels are more intermediate or variable (Brady and Weil, 2002). Therefore, with comparatively high DO in groundwater, N_2O can be a dominant product of denitrification. Groffman et al. (1996) reported that even low rates of denitrification can consume significant amounts of NO_3^--N and produce significant amounts of N_2O. Nitrous oxide decreased with increasing depth of groundwater at JC, because in deeper zones autotrophic transformations of NO_3^- to N_2O and from N_2O to N_2 resulted in less N_2O accumulation, where DOC is low but reduced Fe or S minerals act as electron donor. Higher N_2O production and accumulation in subsoil was in agreement with Weymann et al. (2010) and von der Heide et al. (2009) who reported elevated N_2O production and accumulation up to 2 to 3 m bgl which were considerably higher than the deeper layer of 6.5 to 7 m bgl. Deurer et al. (2008) identified a zone of considerable N_2O accumulation close to the groundwater surface. However, N_2O in groundwater can be produced in situ or can be leached from surface soils. Muhlherr and Hiscock (1998) reported that the unsaturated zone was a source of N_2O for British limestone aquifers. Weymann et al. (2009), von der Heide et al. (2009) and Weymann et al. (2010) concluded that N_2O accumulation resulted from in situ production in groundwater. Therefore, when

measuring denitrification in groundwater, there is concern whether the denitrification products are produced in situ or if they have been leached from surface soils (Groffman et al., 1998). To answer this question, in situ denitrification rate was measured using push-pull method. In addition, the possibility to leach N_2O to GWT from surface soils is very low because it is evident that N_2O transforms to N_2 in subsoils (Clough et al., 2005), which is also in line with the subsoil denitrification results in this study (Chapter 4).

It was obvious that N_2O production was seasonally variable with higher concentrations in February to May when there was high water recharge and low groundwater temperature which might increase the DO concentration in groundwater (Deurer et al., 2008). Difference in volume of groundwater recharge could be a reason for the variability of N_2O over time (von der Heide et al., 2008). von der Heide et al. (2009) reported a strong negative correlation between GWT fall and N_2O flux. Similarly lower shallow groundwater N_2O concentrations of (0.329 mg L^{-1}) in October and higher concentrations in May (0.611 mg L^{-1}) were reported by von der Heide et al. (2008). Recharge can increase groundwater DOC and NO_3^--N concentration and hence increase heterotrophic denitrification resulting in an accumulation of N_2O (Davidson, et al., 1993). Groundwater N_2O concentrations showed higher spatial variability than temporal which was in line with the findings of von der Heide et al. (2008). The mean CV values indicated high spatial variability of catchment scale groundwater N_2O concentrations (mean CV 121, 180, 162 and 82%, respectively, at JC, SH, OP and DG). High spatial variability of groundwater N_2O concentrations were also reported by other researchers e.g. CV 219% (von der Heide et al., 2008), 258% aquifer scale and 19 – 109% transects scale (von der Heide et al., 2008).

6.4.7.2 Groundwater complete denitrification: Excess N_2

Excess N_2 concentrations in these study sites (median between 0.40 mg N L^{-1} at DG and 2.30 mg N L^{-1} at JC) were comparatively lower (median between 2.08 – 7.97 mg L^{-1}) than Weymann et al. (2008). The maximum values between 5.60 and 8.69 mg N L^{-1} at JC and SH sites were measured with RP values between 0.97 and 0.99 at interface and bedrock zones which were slightly higher than Böhlke et al. (2007), who found the highest value of 5.88 mg N L^{-1} excess N_2 in NO_3^- contaminated groundwater in Nebraska, USA. The high RP values were consistent with very low NO_3^- concentrations indicating the occurrence of complete denitrification. The higher excess N_2 at JC and SH indicated that complete

denitrification required proper anaerobicity e.g. DO <2.0 mg L^{-1}, Eh <100 mV and an electron donors like DOC or reduced Fe/S minerals. Conversely, low excess N_2 can be produced under high DO (7.1 - 8.7 mg L^{-1}) and Eh (150-250 mV) with available electron donors mainly DOC and reduced S and Fe as observed at OP and DG. Unusually high pH (7 - 10) at OP could be another reason of low denitrification as Rust et al. (2000) suggested that above a pH of 8.3, denitrification is arrested. Higher denitrification potential was observed in bedrock than in subsoil ($p<0.05$) at OP. These results were in line with Weymann et al. (2008) who found lower excess N_2 in shallow groundwater compared to groundwater 5 m bgl. They differentiated process zones of heterotrophic and autotrophic denitrification respectively shallow groundwater zone and zone beyond 5 m bgl. Weymann et al. (2010) in a laboratory incubation experiment observed that nitrate removal in the autotrophic zone (6.5 to and 7.0 m bgl) is much more intensive than the shallow zone (1.5 to 4.0 m bgl). Therefore, higher excess N_2 concentrations and lower N_2O/NO_3^- concentrations in the deeper groundwaters revealed that these zones are an active sink for N_2O and NO_3^--N resulting in complete reduction. Blicher-Mathiesen and Hoffman (1999) reported an effective NO_3^- removal in a riparian subsurface fen without N_2O accumulation. DOC concentrations were consistently higher in subsoil than other two zones which indicate that DOC is not the only electron donor in deeper groundwater zones rather Fe, Mn and S minerals also act as potential electron donors releasing Fe^{2+}, Mn^{2+} and SO_4^{2-}. A strong positive correlation between excess N_2 and SO_4^{2-} concentrations was observed at two sites (JC and SH) indicating the presence of Fe and S containing minerals for autotrophic denitrification. Kölle et al. (1985) and Weymann et al. (2010) postulated that high NO_3^- removal in the autotrophic denitrification zone is most likely caused by practically anoxic conditions and high reactive microcrystalline pyrite components.

Temporal variation of excess N_2 was low with some seasonality, higher concentration in July to October. This period had low groundwater recharge, lower DO and NO_3^--N occurrence which promote denitrification. Excess N_2 was lowest during the main recharge period (Nov-Dec). A similar pattern of NO_3^--N removal in riparian groundwater was observed by Maître et al. (2003) who found highest NO_3^- removal in spring and lowest in winter. Highest denitrification in August to October was also in good agreement with Gumiero et al. (2011), who measured highest denitrification during summer and autumn in shallow groundwater in Italy. Curie et al. (2009) showed remarkable NO_3^- retention during the summer and autumn period in the hyporheic zone, which cannot be explained by a simple mixing of waters coming from the river and the chalky hillsides, and was attributed

to denitrification. The spatial variability is more pronounced than the temporal showing groundwater as a heterogeneous system. Groffman et al. (1993) and Nelson et al. (1995) measured denitrification across a slope from ridge-top to the soil-stream interface relative to upslope. Groffman et al. (1993) attributed the consistently higher levels of denitrification in a poorly drained toe-slope position compared to a well-drained ridge.

6.4.7.3 Contributions of groundwater to atmospheric N_2O emissions

N_2O mole fraction $N_2O/(N_2O + \text{excess } N_2)$ in subsoil at OP and in bedrock at DG was very high (0.09 and 0.16) but it is not surprising because excess N_2 concentrations in these depths was very low due to unfavourable hydrochemical conditions which resulted in an incomplete denitrification. High N_2O mole fraction can occur because of low denitrification potential with the prevalence of high NO_3^- concentrations. Magalhaes et al. (2003) showed an increase in N_2O/N_2 ratio (0.11 - 0.34) due to an addition of 0 - 4 mg $N\ L^{-1}$, coupled with a decrease in the denitrification efficiency. These results are similar to what was observed in this study. Interestingly, mole fraction at OP significantly dropped from 0.19 in subsoil to 0.06 and 0.05 at interface and in bedrock which indicates that N_2O concentration reduced dramatically while passing through soils along its flow path. Lower mole fraction indicated complete denitrification resulting in termination of denitrification path way, benign N_2. The N_2O mole fractions in the present study compares with Well et al. (2001) who estimated mole fractions of 0.07-0.32 and 0.06-0.08 in 15-35 and 5-100 cm depths of shallow groundwater under Gleyic Luvisol, respectively.

A higher range of mean EF across sites in Irish groundwater (0.0032 at DG to 0.0586 at SH; with all sites mean and median values of respectively 0.0150 and 0.0070) implied that the IPCC (2006) default value of 0.0025 (EF5-g) is not fair enough to be representative rather it is closer to the IPCC (1997) default value of 0.015 (EF5-g). Wide range of EF was also reported by Hack and Kaupenjohann, (2002). These results were a similar order of magnitude to 0.00058 - 0.01065 (Weymann et al., 2008), 0.00026 - 0.0370 (Jacinthe et al., 1998) but higher than 0.0065 - 0.0087 (Weller et al., 1994). Therefore, IPCC default value needs to consider the existing hydrogeological conditions because groundwater N_2O is the result of simultaneous production and reduction reactions (Well et al., 2005) and these transformations are the reason why N_2O concentration in groundwater does not necessarily reflect actual indirect N_2O emissions (Holl et al., 2005). Following the method of Weymann et al. (2008), EF estimation looks better and similar to each other (Table 6.1) but

smaller than values estimated by IPCC methodology because this method considers initial NO_3^--N (N_2O+excess N_2+NO_3^--N) concentrations, which Well and Butterbach-Bahl (2010) defined as conceptual emission factor (CEF).

6.4.7.4 Nitrate removal capacity across sites

Initial NO_3^- loading ($Frac_{LECH}$) depended on the local hydrogeochemical conditions and land uses. The DG site land was intensively grazed permanent grassland for dairy production but NO_3^- delivery was comparatively higher than any other sites mainly because of deeper GWT with higher permeability without potential NO_3^- reduction and facilitated NO_3^- accumulation in groundwater. The SH site had the lowest NO_3^- input, under grazed clover/grass grassland low inorganic N application. There was significant NO_3^- reduction through denitrification at JC and SH. The low initial NO_3^- at JC and SH could be due to elevated denitrification in surface soils which was not quantified. At OP and DG sites, soil surface denitrification was lower due to unfavourable environmental conditions (e.g., well drained sandy loam soils). TDN (N_2O+ excess N_2) was similar at JC and SH sites because these sites were similar in their hydrogeochemical characteristics contrasting with OP and DG sites.

The RP showed how much initial NO_3^--N was transformed to N_2O and excess N_2 in groundwater zones, which at SH site resulted in 77% reduction of initial NO_3^- loading. The JC site also showed very high NO_3^- removal efficiency accounting for 46% reduction of the initial NO_3^- loading. These two sites can be recognized as denitrification hot spots reducing NO_3^--N to excess N_2. The other two sites (OP and DG) had comparatively poor contribution in reducing of initial NO_3^- loading (8 and 4%) resulting in high net NO_3^- concentrations. Generally observed NO_3^- concentrations ranged 2 - 20 mg N L^{-1} with concurrent RP values ranging between 0.10 - 0.60 with some exceptions of 1.0 at JC and SH. Toda et al. (2002) estimated NO_3^- reduction of 20% in shallow groundwater (4 m bgl) compared to 33, 74 and 8%, respectively, at JC, SH and OP sites in the subsoil (5 m bgl). Weymann et al. (2008) reported the median values for RP between 0.33 and 0.68 in sand and gravel aquifer in Germany compared to the median values of 0.05 - 0.97 in these study sites (diverse aquifer type ranged sandy to clayey).

6.4.7.5 Denitrification along the transect

The wells JC2B and JC2C showed highest excess N_2-N and no NO_3^--N, possibly because of the occurrence of higher denitrification than other wells. The reasons behind the high denitrification in these wells are the supply of higher DOC via dirty water irrigation, which had high biochemical oxygen demand (BOD; section 7). In wells JC3A, JC3B and JC3C, even they are in the middle of transect, NO_3^--N concentrations were higher than other wells. This can be related to the ambient hydrogeochemical conditions, being with lower DOC and higher DO and Eh (Appendix 11). It appears therefore, different hydrogeochemical conditions characterize the NO_3^--N and excess N_2-N distributions across the catchment from top gradient towards the bottom along groundwater flow paths. However, a general trend of increasing NO_3^--N retention consistent with increasing excess N_2-N production was observed along groundwater flow paths from the top gradient towards the bottom of the field which is supported by past researches (Groffman et al., 1993; Burt et al., 1999). A remarkable phenomena of these distributions are that it generally suggests a mass balance of initial NO_3^--N equivalents (i.e., N_2O+excess N_2+ambient NO_3^--N) fall in the range of 3.5-7.2, 3.2-9.5 and 2.7-8.5 mg N L^{-1} at JC in subsoil, interface and bedrock, respectively. At SH, this ranges from 2.2-3.1, 2.2-4.5 and 2.5-3.4 mg N L^{-1} in subsoil, interface and bedrock, respectively. At OP site, this ranges from 9.3-13.1, 10.3-13.1 and 12.6-13.9 mg N L^{-1} in subsoil, interface and bedrock, respectively. At DG site, this distribution ranged from 7.9-18.3 mg N L^{-1}. The discrepancies in the mass balance is that N_2 may not remain completely dissolved in groundwater (Beller et al., 2004) or may be diffused upwardly from groundwater to the atmosphere. There are evidence of upward diffusion of N_2O from shallow groundwater to the atmosphere (Weymann et al., 2011) and from subsoils (80 cm depth) to surface soil (Clough et al., 2005), which indicates a possibility of dissolved N_2 diffusion from groundwater to the atmosphere. However, DG site there was only negligible amount of excess N_2, so the N variations in the N balance can be due to the spatial variations in N input, hydrologic regimes e.g., bypass flow, caves, and fractures, or locations in the field e.g., near septic tank or farm shed etc. These findings again support the past reports on the low NO_3^- delivery to the riparian zones of high-elevation watersheds (Sueker et al., 2001; Sickman et al., 2003) and eventually to the headwater watersheds (Sickman et al., 2003). But NO_3^- reduction in riparian zones depends on its width and other hydrogeochemical conditions (Mayer, 2005).

6.4.7.6 Abundances of denitrifier functional genes in groundwater

Groundwater functional gene copy concentrations (GCC) were found in every site and well with similar concentrations. However, the bacteria and the *nir* genes were found consistently higher in subsoil zone but the *nosZ* were higher in interface and bedrock zones. The abundance of denitrifying community is generally assumed to be ubiquitous. The denitrifying genes are reported to be widespread in phylogenetically distant organisms (Linne von Berg and Bothe, 1992), in surface water, soil and groundwater (Beauchamp et al., 1989), 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) but their expressions required favourable environmental conditions. The results in present study are in agreement with Cavigelli and Robertson (2000) and Holtan-Hartwig et al. (2000) as the abundance of denitrifying genes are similar in all wells and sites but their expressions was controlled by several hydrogeochemical conditions which in turn controlled the denitrification processes.

6.4.7.7 Relationships between groundwater denitrification and existing environmental conditions

Deeper unsaturated zones (USZ) have higher N_2O accumulation than shallower because groundwater with deeper GWT is consistent with higher groundwater Eh, DO and NO_3^- (Table 8.2). Rosenblatt et al. (2001) and Gold et al. (2001) found that in glaciated watersheds in Rhode Island sites with hydric soils had groundwater NO_3^- removal rates greater than 80%, whereas sites with nonhydric soils, which have steeper slopes and greater depth to the GWT had NO_3^- removal rates less than 30%. Saturated permeability is (K_{sat}) positively correlated with N_2O because it resulted in lower residence time. Moreover, high K_{sat} coupled with high DO (Tsushima et al., 2002). DO and Eh increase N_2O within the range of our study data (0.3 – 10 mg L^{-1} and <100 – 200 mV respectively, for DO and Eh) because they increase the aerobicity and thus reduce the denitrification potential giving rise to a higher N_2O accumulation. Intermediate DO (<3.5 mg L^{-1}) can accumulate N_2O in groundwater (Deurer et al., 2008). Because denitrification can occur after DO began disappearing in the pore space, in spite of the presence of DO in bulk groundwater (Nakajima, 1979; Tsushima et al., 2002). Higher NO_3^- concentration coincides with higher N_2O accumulation because it inhibits its further reduction to N_2 (Blackmer and Bremner, 1978; Simek and Cooper, 2002; Von der Heide et al., 2008). DOC was positively

correlated with N_2O as it increased the heterotrophic denitrification. A positive correlation was observed between N_2O and SO_4^{2-} concentrations in groundwater implying that both DOC and S^{2-} contribute to groundwater denitrification as electron donors but possibly S^{2-} contributes at deeper zones due to DOC limitation. DOC was negatively correlated with excess N_2 but SO_4^{2-} was positively correlated. This again was in line with the agreement that denitrification is favoured when carbon is limiting (Korom, 1992; Kelso, et al., 1997). Autotrophic denitrification processes in the sandy aquifer in north Germany were found only in depths GWT of 2 – 3 m (Böttcher et al., 1992). A weak positive correlation was found between N_2O and *nir* genes (NO_2^- reducing genes) and negatively correlated with *nosZ* genes (N_2O reducing genes). This supports that N_2O production occurs in groundwater but is a very dynamic process that depends on various environmental factors.

A negative correlation between excess N_2 and K_{sat} was observed may be because the lower K_{sat} increases groundwater residence time provides NO_3^- a longer residence time to be reduced. Lower permeability soils and sediments also had lower DO concentration. Tsushima et al. (2002) suggested that groundwater with low DO had an over five-fold longer residence time than that in the rest. Deeper unsaturated zone with high K_{sat} values increase DO in groundwater and eventually decrease denitrification. Vidon and Hill (2004) reported that highly permeable coarse sediments, shorter residence times of groundwater in contact with aquifer sediments may restrict the development of anaerobic conditions and decreased in amount of NO_3^- removal. Excess N_2 increases with decreasing groundwater DO and Eh because low DO and Eh potentially indicate high anaerobicity for complete denitrification to occur. Rivett et al. (2008) identified O_2 and electron donor concentrations and availability as the primary factors governing denitrification in groundwater. Böhlke et al. (2007) observed <1.6 mg L^{-1} DO was required for complete denitrification of NO_3^- to N_2. There were some wells at JC and SH where DO is <2.0 mg L^{-1} and Eh is low (-72 to 100) with comparatively elevated amount of NH_4^+. Therefore, NH_4^+ in groundwater can be a good indicator of anaerobicity and potential 'hot spots' for excess N_2. The negative correlation between DOC and excess N_2 could be due the reduction of available C (DOC) to CO_2 as DOC concentration were low (ca. ~1-3 mg L^{-1}). An exception was in 3 wells at JC which had 8 - 16 mg L^{-1} were recognised possibly due to long term dirty water effluent irrigation. Given the stoichiometry of 1:1.25 between NO_3^- and DOC on a molar basis there must be 25% more DOC than NO_3^- required for heterotrophic denitrification to proceed to molecular N (Korom, 1992). One mg C L^{-1} of DOC is capable of converting 0.93 mg-N L^{-1} of NO_3^- to N_2 (Jorgensen et al., 2004). This also implies that DOC

contributes at the upper zone whereafter at deeper zones S^{2-} contributes to complete denitrification (Frind et al., 1990; Deurer et al., 2008; Von der Heide et al., 2008). The SO_4^{2-} concentration showed positive correlation with excess N_2 because S^{2-} oxidation increases with the progress of denitrification pathway resulting in high SO_4^{2-} concentrations. Denitrification can typically release CO_2 or SO_4^{2-} ions (Rivett et al., 2008). Negative correlation between excess N_2 and NO_3^- concentration interestingly implies that excess N_2 in groundwater increases due to occurrence of denitrification processes that substantially reduce NO_3^- (Vogel et al., 1981; Konrad, 2007; Weymann et al., 2008). Nitrate reduction by iron sulphide or manganese sulphide can release Fe^{2+} and Mn^{2+} ions which were observed in 40% of the wells under study. Numerous researchers have suggested autotrophic denitrification with S^{2-} (Bottcher et al., 1990; Postma et al., 1991; Tesoriero et al., 2000; Weymann et al., 2010). Oxidation of sulphur compounds therefore releases Fe^{2+} (Kolle et al., 1985) and provides a viable alternative electron donor in carbon-limited systems (Moncaster et al., 2000; Broers, 2004). Grimaldi et al. (2004) summarized that NO_3^- reduction coupled with the oxidation of sulphur in pyrite can occur in a reaction mediated by the bacterium *Thiobacillus denitrificans*. Groundwater conditions controlling NO_3^- reduction and N_2O emissions need for spatial prediction to help spatially target mitigation measures.

6.4.8 Conclusions and recommendations for natural attenuation

Groundwaters in Irish agroecosystems have the potential for the biogeochemical NO_3^- reduction via denitrification but it shows a large variability between different agricultural sites due mainly to their diverse hydrologic (e.g. K_{sat}, changes in GWT etc.) and geochemical (redox chemistry i.e. DO and Eh; multiple electron donors like DOC and iron sulphide/manganese sulphide, NO_3^- concentration, pH etc.) environments. Denitrification is an important pathway to reduce NO_3^- in groundwater. Denitrifier functional genes are ubiquitous but their expressions depend mainly on groundwater hydrogeochemistry. The denitrification results revealed that subsurface denitrification is not limited to shallow groundwater but also had the potentials to a greater depth. Mean N_2O emission factors were close to the IPCC (2006) default value in sandy aquifer but were significantly higher in poorly drained sites. Indirect N_2O emission from groundwater is important. Under favourable environmental conditions N_2O was further reduced to environmentally benign N_2 along groundwater flow paths. Indirect N_2O emissions uncertainties can be further reduced through increased spatial integration. Hydrochemical, microbial and denitrification

results in few wells at JC shows that dirty water irrigation practices can create a denitrification 'hot spots' in groundwater causing 100% reduction of NO_3^-. The log-transformed Eh, NO_3^--N and DOC are the main drivers of groundwater N_2O and log-transformed DO, Eh and temperature are the major predictors of excess N_2. Denitrification potential across national and international boundaries can be mapped to make a better N management for avoiding the serious environmental and health hazards of reactive N. Production of higher excess N_2 in the down slope of field suggested that riparian zones can be an important sink for agricultural NO_3^- that needs to be studied with regards to its hydrogeochemical conditions, width and plant diversities.

6.5 Determination of In Situ Denitrification Capacity Using Push-Pull Method

6.5.1 Background

Analysis of dissolved N_2O and N_2 in groundwaters from subsoil (5 m, bgl), bedrock-interface (12 m bgl) and bedrock (20 m bgl) underlined that denitrification is an important NO_3^- removal pathway across shallow to deeper groundwaters. However, when measuring denitrification in groundwater, there is concern if denitrification end products are produced in situ or if they have been transported from surface soils (Groffman et al., 1998). Unfortunately, the denitrification process is difficult to measure, and existing methods are problematic for a variety of reasons: high background N_2 and out gassing of dissolved gases (Groffman et al., 2006). The in situ NO_3^- push-pull method was used by several researchers to determine in situ denitrification rates in shallow groundwater (1 to 3 m bgl) where a single mini-piezometer (0.008 m OD; 0.02 m screen length) was used for both injecting (^{15}N + conservative tracers), incubation (5- 72-h) and pumping back groundwater (Addy et al., 2002; Kellogg et al., 2005). Measuring denitrification in deep groundwaters is challenging because of the difference in their hydrologic and hydrogeological environments resulting in greater physical depletion of injected solution via dilution and advective dispersion. The single well push-pull method was assessed to quantify denitrification capacity and DNRA rates in shallow to deeper groundwaters at JC and OP sites. The objectives of this section were to (i) assess in situ push-pull method for quantifying denitrification and DNRA in deeper groundwaters; (ii) determine the in situ denitrification capacity; (iii) estimate N_2O mole fractions (N_2O/N_2O+N_2); and (iv) examine factors controlling the observed spatial trends.

6.5.2 Description of In Situ Push-Pull Method

In situ push–pull method (Addy et al., 2002; Kellogg et al., 2005) was adapted to estimate in situ denitrification rates in shallow to deeper groundwater zones. Push-pull method comprises two steps: (i) push-pull pre-test, injection of previously collected groundwater amended with a conservative tracer (Br^- as KBr) into a single well, followed by the extraction of the groundwater mixture from the same well after an incubation period to have insights into recovery as well as sufficient drifting time of injected solutions for microbial denitrification to occur; and (ii) NO_3^- push-pull test, injection of previously collected groundwater amended with a conservative tracer and ^{15}N-enriched NO_3^-, followed by the extraction of the injected solution after an incubation period (fixed by push-pull pre-test) and analysed for N_2O and N_2 produced via microbial denitrification during the incubation period. Insights into recovery and drifting time are required to fix incubation time that will ensure maximum recovery as well as sufficient drifting time for the expressions of denitrifier activities to detect denitrification gases (N_2O and N_2). In the NO_3^- push-pull test, 20 L (grassland) to 30 L (arable land) groundwater solution was injected into a well and then pulled from the same well after incubating for a pre-defined period. The injection and pumping back of groundwater solution were performed; respectively using a peristaltic pump (Model 410, Solinst Canada Ltd.) and a Grundfos pump (Model MP1, Grundfos, Fresno, CA, USA) with Teflon outlet. Peristaltic pump was not used in pumping water because it was unable to pump water deeper than 6 m bgl. Higher amount of groundwater was used in arable site than grassland site because volume of gravel pack around the screen and permeability of aquifer were higher in former site than later. The injected groundwater solution was prepared with the targeted (20-30 mg N L^{-1}) ^{15}N-enriched (50 atom %) NO_3^--N (as ^{15}N-KNO_3; purity 99%) and Br^- (20-30 mg Br^- L^{-1} as KBr). Before preparing the solution, groundwater was stored in a cold room at 4° C for maximum 1 week, which changed the ambient DO concentration in groundwater. To adjust the DO concentration to the ambient level, groundwater solution was bubbled with a noble gas, sulphur hexafluoride (SF_6- 98.2%; Cryoservice Ltd., Worcester WR4 9RH, UK), whilst the DO concentration was monitored using a DO probe (Multi 340i/SET, WTW, Germany). The SF_6 and Br^- were used in push-pull test to quantify the recovery of the plume introduced to the well. The injected volume of water was sufficient to cover approximately 250 to 1000 kg of aquifer materials (bulk density= 1650 - 2500 kg m^{-3}, porosity = 3 - 12%) after correcting for the sands and gravel pack around the well. The total amount of aquifer materials covered by the solution was calculated as using:

$$Mt = \{\frac{(Vt - Vg)}{Porosity\ of\ aquifer)}\} * Bd \qquad (Eqn.\ 6.12)$$

where Mt is total mass of aquifer material (kg), Vt is total volume of solution (m^3), Vg is volume of gravel pack (m^3), and Bd is bulk density (kg m^{-3}). Prior to injection, the groundwater was amended with ^{15}N-enriched nitrate and Br^-. Then, this amended solution was adjusted to ambient dissolved oxygen (DO) concentrations to mimic aquifer conditions by bubbling SF_6 gas through the dosing solution. We used relatively brief incubation periods (i.e., 4 to 6 h) to optimize recovery of the introduced plume (about 20-90% of its previous concentration). Two conservative tracers, gaseous tracer SF_6 and soluble anion Br^-, provided insight into the recovery of the introduced plume.

6.5.3 In Situ Push-Pull Pretest

Addy et al. (2002) and Kellogg et al. (2005) found in situ push-pull test as a robust and promising test to investigate the in situ denitrification rates in shallow groundwater (up to 3 m depth) using mini piezometers, but in the deeper groundwater zones it was more challenging due to the complex hydrogeological settings and uncertainty of recovery of injected solutions (Buss et al., 2005). The method (Addy et al., 2002; Kellogg et al., 2005) was modified to investigate the in situ denitrification rates in deeper groundwater zones. Prior to the in situ NO_3^- push-pull study, an in situ conservative tracer push-pull pre-test was conducted with trial and error approach to ensure substantial recovery as well as sufficient drifting time of injected solutions for microbial denitrification to occur. Total 20 and 30 L of groundwater was collected from each piezometer respectively, at JC and OP. The volume of water to be injected depended on the volume of water in the gravel pack around the well screen section and the amount of aquifer materials to be covered for denitrification rate investigation. The ground water solution prepared with 20-30 mg Br^- L^{-1} as KBr was pushed into the same well via a peristaltic pump. The amended dosing solution was sampled during the push phase to obtain the undiluted concentration of Br^- (*Co*). The plume was left in the ground for 6-h. After the incubation period, two to three times the dosing volume was pulled taking samples at every 2 L intervals. The Br^- extracted from ground was analysed to determine the recovery of the tracer at each sample interval. Experimentally, 20-30 L of groundwater interacts with a large volume of aquifer material (250 - 1000 kg) (bulk density = 1650 - 2500 kg m^{-3}, porosity = 0.03 - 0.12) and takes feasible time to be pushed and pulled back without changing the hydraulic gradient

around the well. The push-pull pretest or NO_3^- push-pull test was conducted in two wells each day. After at least 1 week, the pretested well was resampled and analyzed for Br^- to ensure that tracer concentration was at ambient level before conducting another pre-test with a shorter incubation period if the original pretest recovery was poor or before conducting the in situ nitrate push–pull test. If SF_6 concentrations were still above ambient levels, further waiting until ambient concentrations were reached was essential. At JC and OP sites 6-h incubation was carried in October 2010, and December 2010, respectively.

6.5.4 In Situ $^{15}NO_3^-$-N Push–Pull Test

In situ nitrate push-pull tests were carried out in S, I and B at JC with three replications and at I and B at OP with two replications (no water in OP in S). To prepare for the in situ nitrate push–pull tests, bulk quantities of groundwater (JC: 20 L; OP: 30 L) was collected from the test well and stored in a plastic container (15 L carboy). Ground water was stored at 4°C (maximum of 1 week) until the commencement of push–pull test. Each dosing solution (20-30 L per well) consisted of ambient ground water enriched with 20-30 mg L^{-1} Br^- (as KBr) and 20-30 mg L^{-1} isotopically enriched (50-atom % ^{15}N; 99%) NO_3^-–N (as KNO_3^-–N). Prior to injection, SF_6 gas was bubbled into the dosing solution to saturate the solutions with SF_6 (approximately 30 min per solution) and lower the DO to ambient levels. The carboy was then capped, filled its headspace with the SF_6 gas mixture, and sealed its vents for transport to the study site. The 20-30 L dosing solutions were pushed into wells over the course of 1-2 h (depending on the permeability) with a peristaltic pump (Model 410, Solinst Canada Ltd.) at very low rates (10 to 15 L h^{-1}) to minimize changes in the hydraulic potential surrounding the well. The dosing solution carboy was maintained under constant pressure through connection to the SF_6 cylinder and avoided contamination with atmospheric air. A small quantity of the dosing solution (targeted 500 ml and measured later in the lab) was left at the bottom of the carboy to measure DO and other dissolved gases and hydrochemistry and ensured that the DO content remained stable. Based on the pre-test results, the incubation period was set at 4 - 6-h. After the incubation period, 60 -90 L of ground water was pulled from each well. Ground water from the well was pumped slowly (10 to 15 L h^{-1}) at the same rate it was pumped into the well using a Grundfos pump (Model MP1, Grundfos, Fresno, CA, USA) to avoid generating gas bubbles within the Teflon tubing (gas-impermeable) and to maintain the same hydraulic head. Dissolved gases were extracted from ground water samples as described below. All ground water samples were stored at 4° C and analysed within 1 week.

6.5.5 Conservative Tracer Recovery Estimates

For each well, the recovery or *C/Co* of the conservative tracers was calculated where *C* was the pulled ground water concentration following incubation and *Co* was the original pushed ground water concentration (Freeze and Cherry, 1979). Relative concentration profiles were created by plotting the *C/Co* versus the normalized plume volume (cumulative pulled volume when the sample was collected/total pushed volume).

6.5.6 Dissolved Gas Analysis

Dissolved gases N_2, N_2O, CO_2, CH_4 and SF_6 gases in ambient, pushed, and pulled samples, were extracted using the phase equilibration headspace extraction technique (Lemon, 1981; Davidson and Firestone, 1988). Groundwater samples were collected with a syringe attached to an air-tight sampling apparatus made of stainless steel tubing connected to the Grundfos pump (Model MP1, Grundfos, Fresno, CA, USA). Groundwater samples (120 ml) were injected into an evacuated serum bottle (160 ml) and the headspace (40 ml) was filled with high-purity helium gas (*He*: water ratio = 1: 3; v/v). After shaking for 5 min on a Gyrotory shaker (Model G-10, New Bruns- wick Scientific Co., USA) and a standing period of 30 min, headspace samples were then taken for analysis of concentration and ^{15}N enrichment in 12 ml exetainers (Labco Ltd., Wycomb, UK). Concentrations of N_2O and SF_6 gases were analysed by electron capture gas chromatography, and CO_2 and CH_4 by thermal conductivity detector and flame ionization detector, respectively (CP-3800, Varian, Inc. Switzerland). Concentrations and isotopic composition of ^{15}N-N_2O and ^{15}N-N_2, ^{15}N-NH_4^+ were determined on a dual inlet isotope ratio mass spectrometer (Stable Isotope Facility, UC Davis, Davis, CA) as described by Mosier and Schimel (1993).

6.5.7 Denitrification and DNRA Rates Calculations

Only samples taken from the plume core (determined based on the maximum recovery of conservative tracers) were used in denitrification rate calculations. To calculate the masses of N_2O–N and N_2 gases (μg) in headspace extraction samples, equations and constants provided by Tiedje (1982) and Mosier and Klemedtsson (1994) were used. The total mass of N_2O–N or N_2 was transformed to the mass of $^{15}N_2O$–N or $^{15}N_2$ by multiplying it by the respective ^{15}N sample enrichment proportion (ratio of pulled atom % of the dissolved N_2 and N_2O-N to pushed NO_3^- –N atom %, both corrected for ambient atom %). Sample

$^{15}N_2O$–N and $^{15}N_2$ gas production rates were expressed as μg N kg^{-1} d^{-1} (total mass of $^{15}N_2O$–N or $^{15}N_2$ per volume of water pulled/ [dry mass of soil per volume of water pulled x incubation period]). Each pulled sample represented 1 L of ground water that occupied 13.5 - 50 kg of aquifer materials (bulk density =1650 - 2500 kg m^{-3}, porosity = 0.03 - 0.12). The incubation period was defined as the length of time between the end of the push phase and the start of the pull phase since the plume core would consist mostly of the later injected ground water. Denitrification rates were the sum of $^{15}N_2O$–N and $^{15}N_2$ generation rates. The DNRA rates were calculated similarly as denitrification rates from the production of ^{15}N-NH_4. All samples used in denitrification calculations contained at least 8 mg L^{-1} NO_3^- –N to ensure that the denitrification rate estimates were not limited by the amount of nitrate available (Schipper and Vojvodic-Vukovic, 1998). Denitrification rates and DNRA rates were derived from the total concentration of $^{15}N_2O$–N and $^{15}N_2$ gases and ^{15}N-NH_4^+ obtained through mass spectrometer analysis and were of finer resolution (at the μg L^{-1} level) than Br^- and NO_3^- –N data (at the 0.5 mg L^{-1} level) obtained by ion chromatography.

6.5.8 Statistical Analyses

The measured denitrification rates were approximately log-normally distributed. Therefore, non-parametric test Kruskal-Wallis H tests were performed to determine significant differences in groundwater denitrification rates among depths within each site. Paired *t* tests (Ott, 1993) were performed to determine significant differences in recovery (*C/Co*) between Br^- and SF_6. Mann–Whitney U tests (Ott, 1993) were performed to determine significant differences in denitrification rates observed at JC and OP. Spearman Rank Order correlations were performed to determine significant correlations between groundwater denitrification rates and DO, Eh, NO_3^--N, DOC and K_{sat}. All statistical analyses were performed on GenStat version 13 (VSN Intl Ltd., UK).

6.5.9 Results

6.5.9.1 Hydrogeochemical characteristics and land use

Groundwater ambient physico-chemical properties related to denitrification contrasted between the two sites (Table 6.3). Mean NO_3^--N concentrations were significantly different between sites ($p<0.001$). NO_3^--N concentrations were significantly higher ($p<0.01$) in

subsoil than that at bedrock-interface and bedrock at JC, but were similar among depths at OP. Groundwater pH was near neutral in all depths at grassland, but was higher in bedrock than at bedrock-interface at OP (Table 6.3). Reduced Fe concentration was higher at JC than at OP which indicated possible a release of Fe (II) from pyrite or other sulphur compound in groundwater beneath JC site. DO concentration at JC ranged from 1.3-1.9 mg L^{-1}, whilst it ranged from 4.1-6.2 mg L^{-1} at OP. DOC at grass and arable sites ranged from 1.0-3.5 and 0.7-0.8 mg L^{-1}, respectively. Interestingly, DOC increases with depths at JC, whereas DO decreases with depths at both sites. A decrease in DO with depths indicated its physical attenuation by dilution or microbial consumption. The Eh, being lower at JC (25-94 µS cm^{-1}) than at OP site (107-163 µS cm^{-1}), was within the favourable range for denitrification to occur. The OP had higher aquifer saturated hydraulic conductivity coupled with deeper GWT than the JC (Table 6.3). In these aquifers, hydraulic conductivity (k_{sat}) at both sites decreased with the increase in groundwater depth (Table 6.3).

Table 6.3Ambient hydrologic and hydrochemical properties; values are means ± SEM, n = 2 (OP) or 3 (JC)

Depth	NO_3^--N	DOC*	DO*	Fe (II)	S^{2-}	Eh	GWT*	K_{sat}*
	----------mg L^{-1}----------			-------(µg L^{-1})-----		(mV)	(m, bgl)	(m d^{-1})
JC								
Subsoil	4.7±1.6	1.0±0.1	1.9±0.1	12±4	26±0.06	94±28	1.8±0.1	0.008±0.002
Interface	2.0±1.8	3.5±2.3	1.3±0.4	48±27	21±0.06	25±62	2.9±0.9	0.024±0.004
Bedrock	2.9±1.3	3.4±2.7	1.6±0.1	14±13	24±0.05	47±43	3.4±1.0	0.030±0.005
OP								
Subsoil; no water in the well								
Interface	10.4±0.3	0.8±0.2	6.2±0.8	4.8±0.7	24±0.05	163±5	4.6±0.1	0.053±0.003
Bedrock	12.6±2.5	0.7±0.2	4.1±1.4	2.7±1.0	18±0.05	107±39	5.1±0.1	0.123±0.003

*DO is dissolved oxygen; DOC is dissolved organic carbon; GWT is groundwater table; K_{sat} is saturated hydraulic conductivity

6.5.9.2 In situ push-pull tracers (Br^- and SF_6) recoveries

The predetermined k_{sat} value in each well provided an insight into the incubation time (mean 0.009 m d^{-1} ± 0.002 (standard error, SE) at JC; mean 0.049 m d^{-1} ± 0.008 at OP) for in situ pretest. However, repeated trials of push-pull pretest revealed that incubation time significantly influenced the tracer (Br^-) recovery ($p<0.001$). Reducing the incubation time increased tracer recovery from 9-30% for the 12-h incubation to 30-80% for the 6-h

incubation. Incubation times <6-h was not tested (except one well at JC which had high permeability) because shorter duration for expressions of denitrifiers may not provide sufficient end products of denitrification (N_2O and N_2) to be detected in groundwater as these gaseous products could further be diluted and dispersed. Tracer recovery for in situ nitrate push-pull test (Br^- and SF_6) did not differ significantly ($p>0.05$) neither between depths of groundwater nor between sites (Figure 6.19a and b). Mean recovery in the core plume outside the gravel pack for Br^- at JC were 49, 46 and 43% (Figure 6.19a), respectively in S, I and B which was 36 and 26% at OP, respectively in I and B (Figure 6.19b). There were no significant differences ($p>0.05$) between Br and SF_6 recovery at each site.

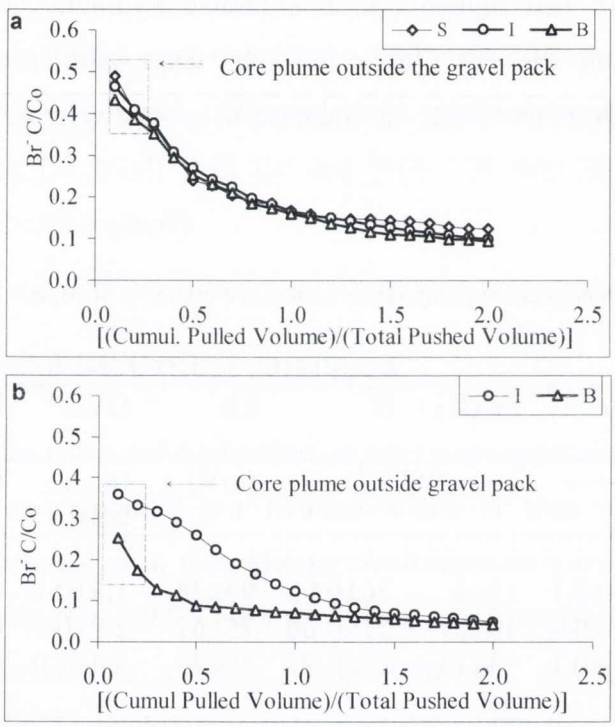


Figure 6.19Relative concentration profiles of conservative tracer (Br^-) in (S), subsoil; (I), interface; and (B) bedrock from the 6-h in situ nitrate push-pull test at JC (a) and at (I), interface; and (B) bedrock at OP (b); The term C represents the concentration of the sample pulled from the well. The term C_0 represents the concentration of the solution originally pushed into the well

6.5.9.3 Results of in situ denitrification rates measured by nitrate tracer test

Over the short incubation period (4 or 6-h), nitrate removal via denitrification was detected at both sites. Denitrification rates at JC (mean = 163 μg N kg^{-1} d^{-1}, SE = 153) were significantly ($p<0.05$) higher than that at OP (mean = 3.9 μg N kg^{-1} d^{-1}, SE = 2.0). Considering differences among three different depths, significantly higher denitrification rates ($p<0.05$) were measured at bedrock-interface (I: mean = 469.5 μg N kg^{-1} d^{-1}; SE =

311); than in subsoil (S: mean =10.9 μg N kg^{-1} d^{-1}, SE = 3.5) and bedrock at JC (B: mean = 9.2 μg N kg^{-1} d^{-1}, SE = 5.8) (Figure 6.20a). Similarly, denitrification rates among two different depths at OP were significantly higher ($p<0.05$) at bedrock-interface (I: 6.4 μg N kg^{-1} d^{-1}, SE = 1.8) than in bedrock (B: 1.4 μg N kg^{-1} d^{-1}, SE= 0.4) (Figure 6.20b). The statistical robustness of the difference among two depths at OP is limited by the number of wells (n=2) but it showed a good indication of the difference in mean rates (pers. comm., Dr. Jim Grant, Teagasc). Denitrification rates equivalent to a weighted average of 3.92 and 0.09 mg NO_3^--N L^{-1} d^{-1}, respectively at JC and OP, which accounted for 24.5 and 0.33% of the applied N. Denitrification in S, I and B at JC site equivalent to 0.2, 10.3 and 0.3 mg N L^{-1} d^{-1} which accounted for 1, 65 and 2% of the N input, respectively. At JC, coefficient of variations (CV) for denitrification between wells was 55, 115 and 109% in S, I and B, respectively. At OP, CV was 50 and 47% at I and B, respectively.

The N_2O/ N_2O+ N_2 ratio were significantly higher ($p<0.05$) at OP (mean = 0.18, SE = 0.02) than at JC (mean = 0.08, SE = 0.02). Mean N_2O/ N_2O+ N_2 ratios were 0.06., 0.05 and 0.14 in S, I and B, respectively at JC (Figure 6.20a) and were 0.24 and 0.10 at I and B, respectively at OP (Figure 6.20b). In situ production of environmentally benign N_2 was the dominant end product of denitrification that ranged from 86-95% of total denitrification gases at grassland site, at arable land it ranged from 76-90% of total denitrification gases.

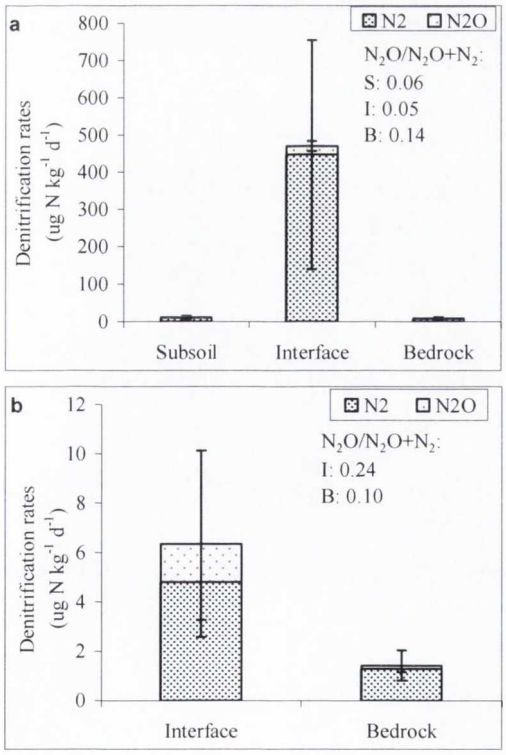


Figure 6.20Mean denitrification rates and N_2O/N_2O+N_2 ratios in three different depths of groundwater (n=3) at JC (a) and in two different depths of groundwater (n=2) at OP (b)

6.5.9.4 Results of in situ rates of DRNA and total NO_3^- reduction

In situ DNRA rate was significantly higher at JC site (mean 3.47 µg N kg^{-1} d^{-1}, SE 1.12) than at OP site (mean 2.42 µg N kg^{-1} d^{-1}, SE 1.02). DNRA contributed respectively to 0.04 and 0.03 mg N L^{-1} d^{-1} which accounted for 0.08 and 0.05% of total injected NO_3^- at JC and OP. Considering the differences among three depths of groundwater at JC and two depths at OP, significantly higher ($p<0.05$) DNRA was observed at bedrock-interface than in subsoil and in bedrock (Figure 6.21). DNRA rates showed high spatial variability with CV values of 14, 84 and 115%, respectively in subsoil, bedrock interface and bedrock at JC and 132 and 141% in bedrock interface and bedrock at OP. Mean total NO_3^- reduction via denitrification plus DNRA was 166.7 and 6.3 µg kg^{-1} d^{-1}, respectively at JC and OP which were equivalent to 4.00 and 0.14 mg N L^{-1} d^{-1} that were accounting for 25 and 0.53% of the applied NO_3^-. However, DNRA contributed to 2.08 and 38.25% of the total nitrate reduction (denitrification plus DNRA), respectively at JC and OP sites.

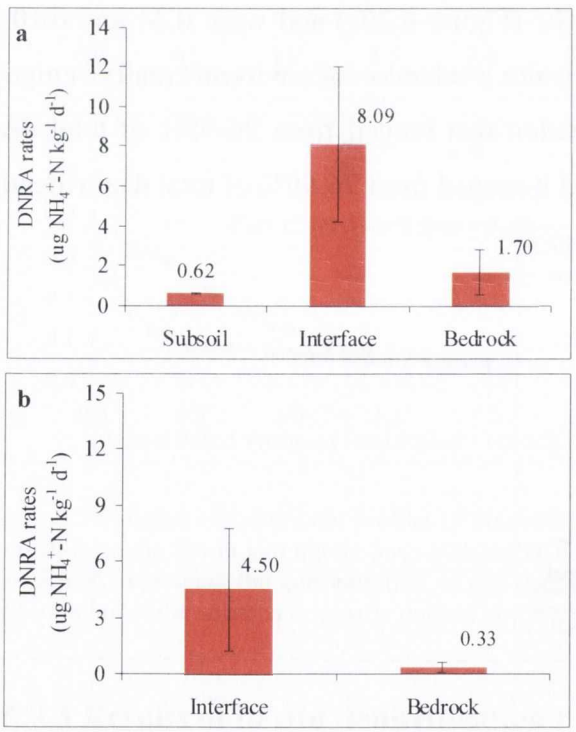


Figure 6.21Mean DNRA rates in three different depths of groundwater (n=3) at JC (a) and at two different depths (n=2) at OP (b)

6.5.9.5 Relationships between denitrification rates and geochemical conditions

Spearman Rank Order correlation between denitrification rates and ambient geochemical properties showed that denitrification rates were negatively correlated individually with ambient DO ($r = -0.52$; $p<0.05$), Eh ($r = -0.52$; $p<0.05$) and NO_3^--N concentrations ($r = -0.69$; $p<0.01$). Saturated hydraulic conductivity showed significant negative correlation ($r = -0.50$; $p<0.05$) with denitrification rates.

6.5.10 Interpretation of recovery of conservative tracers

Estimation of the recovery of tracer is very important for quantifying groundwater denitrification capacity rates and to understand the decline in concentrations of denitrification end products by physical processes like advection, dispersion and diffusion. Similar rates of Br^- and SF_6 recovery indicate that there was no degassing loss of SF_6 during the incubation and sampling. The similarities in the recovery of both tracers also enhance the confidence of estimating groundwater dissolved gases concentrations produced via denitrification during the incubation period. Either of the tracers can be used for investigating groundwater denitrification using the push-pull method. Bromide has been used as the conservative tracer in many riparian groundwater NO_3^- studies (Simmons et al., 1992; Nelson et al., 1995; Starr et al., 1996) and both Br^- and SF_6 (Addy et al., 2002; Clough et al., 2007; Kellogg et al., 2005). However, recovery rates in this study (5-30 m bgl) were relatively lower than Addy et al. (2002) and Kellogg et al. (2005), because they carried out push-pull method at shallower depths i.e. in 0.65 to 1.25 m and 0.65 to 3 m bgl with small screen section length (0.02 m) and diameter (o. d. 0.018 and 0.008 m) with a maximum recovery of 80 and 70%, respectively. But it was within the range of Harrison et al. (2011) who obtained a range of 42-54% recovery in summer and 20-26% in winter at two alluvial wetlands in USA. However, the authors reported an average range of 60-70% recovery of SF_6. Low recovery in our experiment could be due to high advective dispersion and diffusion, and low residence time in these aquifers which have sediments with larger and more connected secondary pores or preferential flow path via fracture/fissure (Buss et al., 2005; Misstear et al., 2009). Sedimentary rocks e.g., Ordovician sediments of sandstones in grassland site and limestones at arable sites showed increased hydraulic conductivity with depth of aquifers (Table 8.3). Solute movement follow piston flow model in subsoil but in bedrock it follows complex pattern of movement because bedrock might have both vertical and horizontal flow paths via fractures developed by glacial

movement. Low recovery in the push-pull test for the denitrification study in deep groundwaters can underestimate nitrate removal rates, because produced gases in groundwater samples can be decreased by dilution or advective dispersion. However, it can be minimized by increasing recovery rates with careful design of the wells (low screen section and sampling at discrete depths) and with some modifications of the method like lowering the pumping rate (reduced hydraulic pressure) and incubation time; and increasing the volume of groundwater solution. There is another reason of underestimation of denitrification rates because NO_2^- and NO production rates are not included in the calculation (Bollmann and Conrad, 1997; Harrison et al., 2011; Istok et al., 1997).

6.5.10.1 Interpretation of groundwater denitrification rates

The results suggest that natural NO_3^--N attenuation via denitrification can be important at JC but not at OP. Considering the depths, denitrification rates were higher at interface than that of other depths estimated at both sites (JC: ranged from 9.2 μg N $kg^{-1}d^{-1}$ in subsoil to 469.5 μg N $kg^{-1}d^{-1}$ at interface; and OP: ranged 1.4 μg N $kg^{-1}d^{-1}$ in bedrock to 6.4 ug N $kg^{-1}d^{-1}$ at interface). Denitrification rates at both sites were within the range of shallow groundwater denitrification rates of Kellogg et al. (2005) (<1 to 330 ug N $kg^{-1}d^{-1}$) and Addy et al. (2002) (2.1 to 123.2 ug N $kg^{-1}d^{-1}$) except our rates at interface at JC (mean = 469.5 ug N $kg^{-1}d^{-1}$; SE = 311 ug N $kg^{-1}d^{-1}$) which is higher than that of the results they obtained. Therefore, these results are suggesting that denitrification can occur in deeper groundwaters only in the favourable conditions (e.g., JC). In addition, these results are in line with the general comments that denitrifier functional genes are ubiquitous (Linne von Berg and Bothe, 1992), because similar concentrations of denitrifier functional genes have been detected in all depths at these experimental sites (section 8.2). These findings are in contrast with the results of Kellogg et al. (2005), who estimated similar denitrification rates among three discrete depths in shallow groundwater (0.65 to 3.0 m bgl). These differences can be attributed to the wider range of aquifer depths (subsoil to deep bedrock) in our study than their study where all depths were within shallow groundwater. Higher denitrification rates at bedrock-interface (10-12 m bgl) are in line with the findings of Weymann et al. (2010) who, from a laboratory incubation experiment, observed that nitrate removal in the autotrophic zone (6.5 to and 7.0 m bgl) is much more intensive than shallow zone (1.5 to 4.0 m bgl). Nonetheless, the mean denitrification rates in bedrock-interface at grassland site indicate that groundwater denitrification can be a potential sink (hot spot) for NO_3^--N at favourable environmental condition before its delivery to the surface waters. Higher

denitrification capacities at bedrock-interface as well as similar denitrification between subsoil and bedrock at JC are therefore clearly indicating that denitrification is not limited to shallow groundwater, rather, it has the potential to reduce NO_3^- in deeper groundwaters. This is in contrast to the assumption of Van Drecht et al. (2003) who developed an empirical model with an assumption that denitrification is zero in deep groundwater.

Denitrification rates showed high spatial variability because groundwater hydrogeological properties that control denitrification capacity are heterogeneous. The coefficients of variation of N_2O concentrations between wells within each site which ranged from 55-115 and 47-50% at JC and OP, respectively, were similar to the coefficients of variation of N_2O production found by other workers, in surface soils e.g., 71-139% (Mathieu et al., 2006), 217% (Yanai et al., 2003), 14-132% (Ishizuka et al., 2005) and in shallow groundwater e.g., 219% (Von der Heide et al., 2008). This indicates that denitrification is likely to be an active process, as it is in top soil, of natural NO_3^- reduction in shallow to deeper groundwaters. Moreover, high spatial variability of N_2O production is consistent with the high spatial variabilities in groundwater DO (CV 120%), Eh (CV 219%) and DOC (CV 98%), suggesting that NO_3^- in groundwater is being processed and these properties can be the key indicators of groundwater denitrification.

Higher N_2O mole fractions at arable site than that of the grassland site (JC: mean 0.08; arable: mean 0.17) might have occurred due to low N_2O reduction rates at this site, because high DO at this site might have reduced N_2O reduction and thus increased its accumulation. Mean N_2O mole fractions in the in situ measurements were comparable to the range of a laboratory incubation results from subsoil denitrification at grassland site with 0.25 to 0.42 in 0 - 10 cm; 0.06 to 0.36 in 45 - 55 cm and 0.04 to 0.24 in 120 - 130 cm depths (Chapter 6). The N_2O mole fraction in this study (0.05-0.24) was well comparable with Harrison et al. (2011) who quantified N_2O/N_2O+N_2 ratios of 0.02- 0.21 in 0.5 m bgl in alluvial wetlands using in situ push-pull method. Mean N_2O mole fraction as calculated at each site implies two possibilities: one is that groundwater could be an important source of atmospheric N_2O when it discharges to surface streams and rivers (Deurer et al., 2008) or diffused upwardly from water table to the atmosphere (Ueda et al., 1993) and another is that N_2O can further be reduced to N_2 (Weymann et al., 2008). Mean mole fractions 0.02 at grassland to 0.09 at arable land from a monthly measurements over the last two years period (2009-2010) in these wells were lower than that of the measurements by in situ push-pull test, because N_2O might have been further reduced to N_2 while passing through

and from the sediments to the streams due to its longer residence times. However, another possible reason of higher N_2O/N_2O+N_2 ratios in the in situ study than that of monitoring results could be the addition of NO_3^- to groundwater by at least 2 times of the ambient concentration, as high NO_3^- concentration can accelerate N_2O production (Scholefield et al., 1997; Blackmer and Bremner, 1978), inhibit N_2O reduction (Simek and Cooper, 2002) and eventually increase the N_2O mole fraction. The monitoring results suggest that denitrification is more complete, resulting in lower N_2O mole fractions, taking into consideration of the travel time through aquifers which can take from months to years at these sites (Fenton et al., 2011).

The reasons of higher denitrification at JC than at OP or more specifically higher denitrification at bedrock-interface at JC that in all other depths can be explained by their contrasting hydrologic and geochemical conditions (Table 8.3). The DO and Eh are lowest at bedrock-interface at JC. The DO, being comparable in all depths at JC, is lower than the OP. The DO and Eh point out the higher anaerobiocity of groundwater and thus increased denitrification rates. Rivett et al. (2008) identified DO and electron donor concentrations and availability as the primary factors governing denitrification in groundwater. Böhlke et al. (2007) observed <1.6 mg L^{-1} DO as required for complete denitrification of NO_3^- to N_2. The higher DO and Eh at OP than at JC suggesting that detection of in situ denitrification may be either very poor or zero under aerobic conditions. The observed denitrification rates, though small at the OP, could be attributed to either deriving from aerobic denitrification (Robertson et al., 1995) or through denitrification occurring in anaerobic microsites (Seitzinger et al., 2006). From groundwater monitoring results of hydrochemistry and dissolved gases (N_2O and excess N_2, called denitrified N_2), higher NO_3^- and lower N_2O and N_2 concentrations were observed at OP that at JC.

DOC enhances denitrification by reducing DO through aerobic respiration, releasing CO_2 and as an electron donor for denitrifier community. Moreover, DOC is not only available to shallow groundwater but also leached out to the deep groundwater as there was no significant decline in DOC with depth 5 to 30 m bgl. Surprisingly no significant correlation between DOC and denitrification capacity rates was observed in this study may be due to the high spatial variabilities in DOC concentration (<1 to >10 mg L^{-1}). In deep groundwaters, however, other electron donors can be of importance as denitrification rates showed positive correlation with reduced Fe, which was the highest at bedrock-interface at JC. It is possibly because of the oxidation of sulphide compounds (bound with Fe) under

anaerobic conditions and thus the release of Fe (II) or Mn (Kolle et al., 1985). Negative correlation between denitrification and ambient NO_3^- concentration implies that low ambient NO_3^- existed in groundwater wells due to occurrence of natural denitrification process that substantially reduced NO_3^- (Konrad, 2007; Vogel et al., 1981; Weymann et al., 2008). As such in groundwater as a steady state reactive system denitrification can consume NO_3^- and release N_2O plus N_2.

Therefore, denitrification at bedrock-interface (469.5 μg N $kg^{-1}d^{-1}$) at JC appears to be a 'hot spot' to NO_3^- removal and substantially reduces surface water NO_3^- delivery and indirect N_2O emissions to the atmosphere. However, denitrification rates at this site in subsoil and bedrock (9.2-10.9 μg N $kg^{-1}d^{-1}$), being significantly lower than that of the bedrock-interface, can still be considered as an important NO_3^- removal process. Conversely, at OP it appeared that the role of denitrification in NO_3^- retention is negligible but its contribution to indirect N_2O emissions can be of importance. The longer flow paths in groundwater could also provide the conditions favouring complete denitrification and reducing atmospheric N_2O emissions. The grassland site was on a 48 ha land that has been cultivated for 35 years and the OP was on a 10 ha field located in a 250 ha area and has been cultivated with spring barley with or without cover crop under conservation and conventional tillage systems.

6.5.10.2 DNRA vs. total NO_3^- reduction in groundwater

The DNRA rates in this study in both sites imply that it can be an important pathway of NO_3^- reduction, but its magnitude in two sites is different may be due the existing hydrogeologic conditions. Nitrogen fixation and DNRA are important mechanisms that add and retain available N in Texas estuaries (Gardner et al., 2006). Rütting et al. (2011) suggested that DNRA can be a significant NO_3^- consumption process in some ecossytems. At JC site, DNRA is favoured in low NO_3^- concentrations coupled with comparatively higher DOC concentrations but at OP site it occurred in high NO_3^- concentrations coupled with low DOC concentrations (Table 8.3). Morley and Baggs (2010) concluded that DNRA is stimulated by carbon but in contrast to this report, Yin et al. (1998) reported that carbohydrate do not support DNRA. Several researchers suggested that DNRA occurs in more reducing conditions (Takaya, 2002; Page et al., 2003) but others showed that DNRA is less sensitive to variable redox conditions (Pett-Ridge et al., 2006). Therefore, both chemolithoautotrophic (coupled with sulphur oxidation) and fermentative (Organic C as

electron donors) DNRA might occurred in these groundwaters. As At OP, DNRA occurred in groundwater with comparatively high DO conditions, it can be noted that DNRA can occur in anaerobic microsites (Rütting et al., 2011), or even in partially aerobic environment if electron donors e.g., metal bound sulphide are available (Simon, 2002). This argument is again supported by Fazzolari et al. (1998), who showed that DNRA is less sensitive to oxygen than denitrification. Though both denitrification and DNRA occurred simultaneously, which were in line with Morley and Baggs (2010). However, denitrification was the dominating process over the DNRA in both sites. Similar results of higher denitrification than DNRA was observed in a NO_3^- contaminated groundwater (Smith et al., 1991) and in estuaries (Gardner et al., 2006). The potential energy required for complete denitrification and DNRA are -2669 and -1796 $kjmol^{-1}$, respectively (Gottschalk, 1986), suggesting that denitrification should favour over DNRA (Rütting et al., 2011). Conversely, Bengtsson and Annadotter (1989) noted similar contributions of denitrification to DNRA in nitrate reducing in groundwater resulting in a total rate of 250 $\mu g\ kg^{-1}\ d^{-1}$. However, NH_4^+ fixation with clay minerals or its further conversion to N_2 coupled with NO_2^- (anaerobic ammonium oxidation-anammox) can cause denitrification as the dominating process particularly in JC site with very low DO where a strong correlation between NO_2^- and NH_4^+ was observed (R^2=0.645).

6.5.11 Conclusions of in situ denitrification capacity

Hydrologic characterization of groundwater aquifer is difficult and thus the push-pull pretest is essential to apply NO_3^- push-pull test. Denitrification capacity rates were determined within a short period of incubation but longer incubation times can increase rates and decrease N_2O mole fractions. A longer incubation than 6-h in these aquifers is not feasible, because it decreases the recovery of ^{15}N-enriched NO_3^- due to physical attenuation and thus might cause underestimation of denitrification rates. However, decreasing the screen length of well, sampling at discrete depths, slow injection rates and low incubation period can be of help to increase the recovery rates. This study showed that push-pull method for groundwater denitrification study using ^{15}N-enriched NO_3^- can be used in shallow and deeper groundwater systems, while improved tracer recovery will definitely give more accurate estimation. The bedrock-interface at JC shows that this zone in groundwater can serve as a 'hot spot' for denitrification to occur. However, at this site the subsoil and bedrock also showed a good of denitrification capacity rates, suggesting

that groundwater denitrification at JC can be a sink for NO_3^-. Denitrification capacity rate does not seem to be a significant NO_3^- removal pathway at OP but its contribution to indirect N_2O emissions should be accounted for global N_2O budget. The strong variation with hydrogeologic conditions suggests predictability and mapping of sites with significant subsurface denitrification capacity. Understanding denitrification capacity in groundwater ecosystems can help formulate a rationale management of N inputs in the agricultural systems and thus help finding solutions to the problems created by the formation and release of reactive N in an ecosystem. These findings show important implications about the natural NO_3^- attenuation capacity of groundwater beneath intensively managed grassland that reduces the risk of NO_3^- delivery to the surface waters. In addition, N_2O mole fractions from in situ and natural measurements indicated that groundwater denitrification can reduce indirect N_2O emissions to the atmosphere. Supplying adequate C sources to groundwater e.g., via C rich farm washes, dirty water irrigation, constructing denitrification reactive barriers along groundwater flow paths can enhance denitrification process. However, in groundwaters with high DO and Eh but low DOC are more vulnerable to nitrate contamination and indirect N_2O emissions.

6.5.12 Potential confounding factors of push-pull method in deeper groundwaters

Hydrologic characterization of groundwater aquifer is difficult and thus the push-pull pretest is essential to apply NO_3^- push-pull test which can be time consuming and expensive. Denitrification rates were determined within a short period of incubation but longer incubation times can increase rates and decrease N_2O mole fractions, but longer than 6-h incubation in these aquifers is not feasible to have approximately 50% recovery. Denitrification rates can be underestimated as NO_2^- and NO are not included in the calculations.

6.5.13 Comparisons between short-term incubation and long-term monitoring results for groundwater denitrification

In situ groundwater denitrification rates measured by ^{15}N tracer test (incubated in situ for 4- 6-h) were compared with the in situ monitoring of N_2O and excess N_2 (monitored over

two years-Feb, 2009 to Jan, 2011). In situ denitrification rates for total denitrification (N_2O+N_2) showed higher NO_3^- reduction than the monitoring data (N_2O+N_2). At JC, the weighted mean concentrations across three depths of groundwater were 3.92 and 2.52 mg N L^{-1}, respectively from in situ incubation tests and in situ monitoring results accounting for 25 and 50% of the total N content of groundwater. It is not surprising because N_2O+N_2 concentrations obtained from monitoring results have been generated over longer time than the in situ incubation time, contributing to a higher fraction of the total ambient N. Even though, fraction of denitrified N over the total applied NO_3^- in the in situ test was lower than the monitoring results, the rate of NO_3^- reduction from in situ test was higher. These results showed an important implication to understand the capacity of groundwater in NO_3^- reduction via denitrification. This is again supported by the N_2O production rates during in situ denitrification where N_2O production rates were higher than the in situ monitoring of N_2O concentrations, as revealed by the $N_2O/(N_2O+N_2)$ ratio (Figure 6.22). Therefore, from the denitrification capacity results obtained by the in situ tracer test, it can be concluded that at the JC site, denitrification rate is either nitrate limited or excess N_2 over the long period is diffusing up from groundwater to the atmosphere through the unsaturated zone. Diffusion of N_2O from groundwater to the atmosphere through the unsaturated zone is reported by Weymann et al. (2010) but diffusion of N_2 is not reported so far. In contrast to JC, the OP site showed lower in situ denitrification rates (0.09 mg N L^{-1}) than in situ monitoring result (0.91 mg N L^{-1}). Percentage of NO_3^- reduced over the total ambient N was also lower from in situ tracer test (0.33%) than in situ monitoring results, which was similar to JC. This contrasting result between two sites signifies that denitrification at OP is not NO_3^- limited and a significant amount of nitrate reduction by denitrification was unlikely, which is opposite to JC. The higher N_2O/N_2O+N_2 ratio from in situ tracer test than the in situ monitoring result therefore suggested that groundwater produces substantial amount of N_2O but these diffuse upwardly to the atmosphere (Weymann et al., 2010) or undergoes to further reduction while passing through and from the landscape to the receptors. In addition, the N_2O concentrations from in situ tracer test showed significant positive correlation ($r = 0.67$; $p<0.01$) with the in situ monitoring of excess N_2. It indicates that the longer is the residence time along groundwater flow paths the higher is the N_2 production as N_2O is being reduced to N_2 constantly.

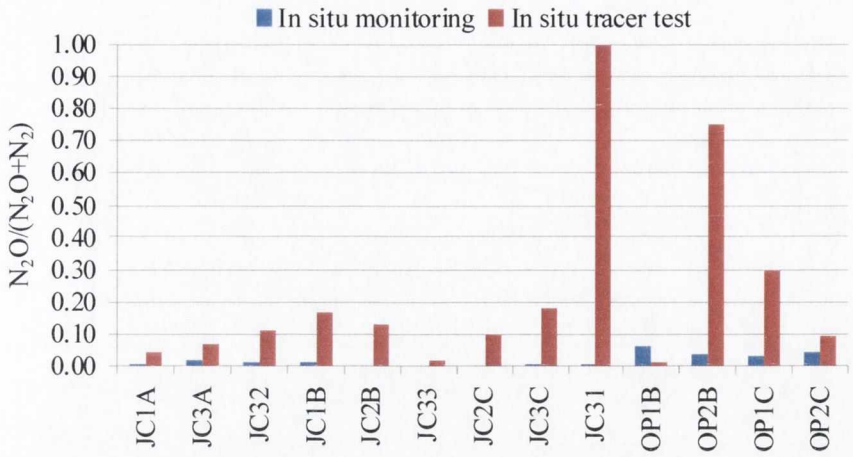


Figure 6.22$N_2O/(N_2O+N_2)$ ratios from in situ monitoring wells and in situ tracer (incubation) test

6.6 In situ denitrification capacity in shallow groundwater beneath spring barley-cover crop rotation

6.6.1 Site and experimental design

In situ denitrification rates were measured in March 2011 using a push-pull method in shallow groundwater (~ 4 m bgl) beneath an arable field (Sawmills Field, 10 ha) at Oak Park Crop Research Centre, Teagasc, Ireland (site description is given in Chapter 3). The total area of the arable land was 250 ha. The Sawmills Field has been cultivated with two different crop rotations: (1) spring barley with cover crop (mustard) and (2) spring barley without cover crop since 2006. The field was well drained with sandy loam top soils overlain sands intermixed with gravels in subsoils. Below the glacial till, carboniferous limestones are present approximately at 10 m bgl. Mean GWT ranged from 1.5 m in winter to 3 m bgl in summer with an annual fluctuation of 2 m. Three wells (PVC pipe; 0.03 m i. d. and 1.0 m screen section) were installed in each plot (3 replications) (Figure 6.23). In Plot 1 wells p.1.2, 1.3 and 1.4 and in Plot 2 wells p.2.2, 2.3 and 2.4 were selected for the incubation study. Wells p.1.1 and p.2.1, respectively in Plot1 and Plot2 were excluded due to lack of sufficient water. The surface runoff and horizontal flow of rainwater in this field was considered negligible due to the free draining nature of soils and subsoils. Therefore, the percolating water through the rooting zone assumed to bear nitrate and other nutrient directly to GWT. Rainfall in the arable field in such high permeable land in Ireland is not reported to exceed the infiltration capacity of soils (Fenton et al., 2008).

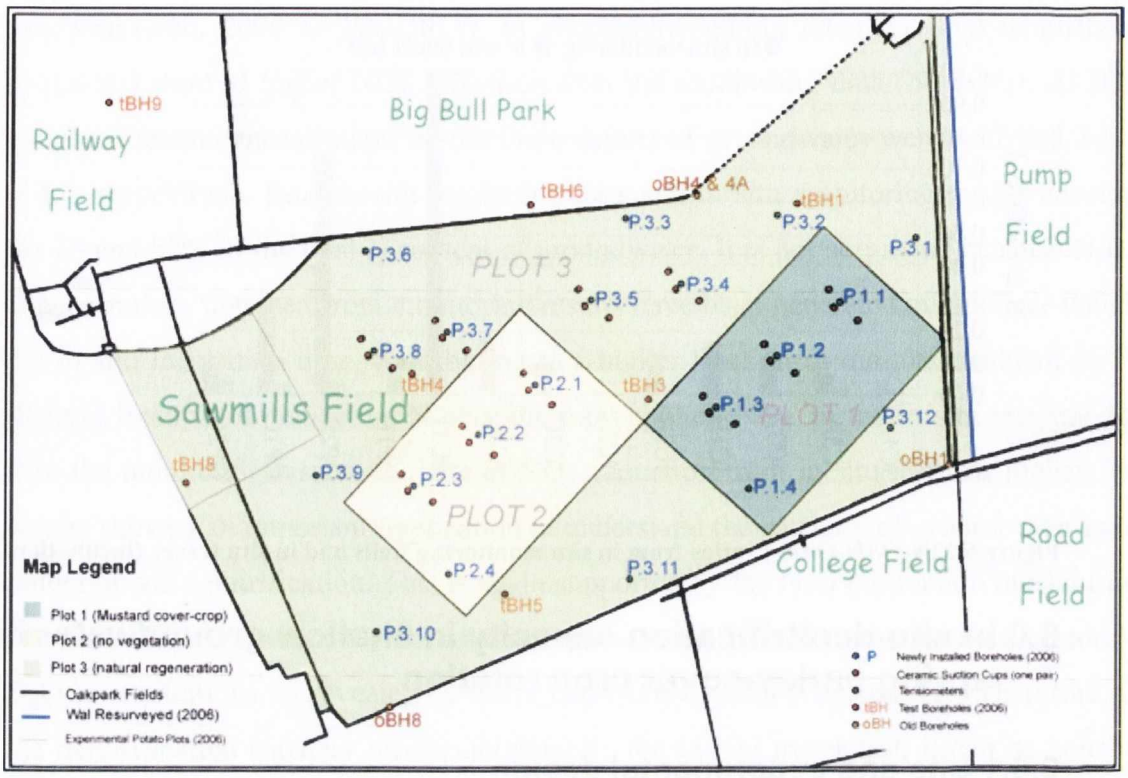


Figure 6.23Location of the experimental field (Sawmills Field) showing selected wells (p 1.1, p 1.2, p 1.3 in PLOT1 and p 2.1, p 2.2, p 2.3 in PLOT2) from a net work of previously installed wells at Oak Park Crop Research Centre showing the two differently managed plots with wells in the respective plots (after Premrov, 2011).

6.6.2 In situ push-pull method

To prepare for the in situ NO_3^- push–pull tests, 10 L groundwater was collected from the test well and stored in a plastic container (carboy). The 10 L groundwater covers approximately 42 kg of aquifer materials (bulk density: 1.65g cm^{-3}; porosity: 0.38%) after correcting for the sands and gravel pack around the well. Each dosing solution (10 L per well) consisted of ambient ground water enriched with 50 mg L^{-1} Br^- (as KBr) and 50 mg L^{-1} isotopically enriched (50-atom % ^{15}N) NO_3^-–N (as KNO_3^-–N). Only one conservative tracer was used here because previous experimentations in different groundwater depths in the same aquifer (section 6.5) suggested that the recovery of Br^- is similar to the recovery of SF_6. Moreover, similar rates of recovery of Br^- and SF_6 were measured in an in situ push-pull test by Addy et al. (2002) whereby they suggested that any one of the tracers can be used. The dosing solutions were pushed into wells over the course of half an hour with a peristaltic pump at low rates (20 L h^{-1}) to minimize changes in the hydraulic potential surrounding the well. A small quantity of the dosing solution (targeted 500 ml) was left at the bottom of the carboy to measure DO and other dissolved gases and hydrochemistry to ensure that the DO content remained stable. Based on the pre-test results, the incubation

period was set at 4 h. After the incubation period, 30 L of groundwater was pulled from each well. Groundwater was pumped from the well slowly (20 L h^{-1}) at the same rate it was pumped into the well using a peristaltic pump to avoid generating gas bubbles within the Teflon tubing (gas-impermeable) and to maintain the same hydraulic head. Groundwater samples were collected in every 2 L interval into 12 ml exetainers (Labco Ltd., Wycombe, UK) for dissolved N_2 and Ar); into 160 ml glass serum bottle for dissolved N_2O, CO_2 and CH_4; into 50 ml plastic tube for hydrochemical properties. Details of groundwater sample storage, dissolved gases analyses, tracer recovery analyses and calculation of denitrification rates were explained in section 6.5.

6.6.3 Statistical Analysis

The measured denitrification rates were approximately log-normally distributed. Therefore, non-parametric test Mann–Whitney U test (Ott, 1993) was performed to determine significant differences in denitrification rates observed in two different cropping systems. However, the obtained differences between land uses were an indication, because the land was not replicated (not feasible in this case) but wells were selected randomly that were scattered at least 50 m from one another along groundwater flow path. All statistical analyses were performed on GenStat version 13 (VSN Intl Ltd., UK).

6.6.4 Results of ambient hydrochemical properties

Nitrate delivery to shallow groundwater beneath the plot with spring barley following cover crop rotation was significantly higher ($p<0.05$) than the plot without cover crop (Table 6.4). The DOC concentrations in groundwater beneath both plots were very low (<1.5 mg L^{-1}). However, it was significantly higher in groundwater beneath cover crop cultivated plot than the no cover crop cultivated plot (Table 6.4). Groundwater hydrochemical properties, being studied e.g. DO, Eh, pH, EC and SO_4^{2-}, were statistically similar in both plots even though the DO and Eh were slightly lower in wells beneath the cover crop cultivated plot than beneath the no cover crop plot (Table 6.4). High DO concentrations (>10 mg L^{-1}) in such aquifer was due to the free draining nature of the sediments because DO equilibrate with the percolating water and quickly reached groundwater without its further consumption.

Table 6.4 Hydrochemical properties in two differently managed arable plots (mean ± SE; n=3)

Treatment	NO_3^--N	DOC	DO	SO_4^{2-}	Eh	pH	EC
	----------mg L^{-1}----------				(mV)		µS cm^{-1}
Spring Barley - Mustard	13.6±2.6a	1.3±0.1a	10.2±0.2a	23.9±2.1a	185±5.0a	7.8±0.1a	441±17a
Spring Barley - No Vegetation	20.2±4.5b	0.9±0.1b	10.7±0.4a	20.5±1.7a	190±5.8a	8.0±0.1a	411±16a

Means with the same letters within each column does not differ significantly between treatments

6.6.5 In situ tracer recovery

The tracer recovery data showed that injected plume was dispersed steadily from the screen section towards the aquifer (Figure 6.24). After the 4-h incubation period, the highest mean recovery, being 59-66% of the injected concentrations of Br^-, was sufficient to calculate the physical losses of the injected nitrate. After pulling 20 L water (2 times the injected volume), the tracer reached almost to the ambient level. The concentrations of dissolved gases in groundwater collected from the initial 6 L (plume core; 41-66% of recovery) were accounted for calculating denitrification rates to minimise the effects of uncertainty of estimation due to physical dispersion and diffusion. Another implication of tracer recovery is that injected plume was dispersed uniformly across the aquifer materials even though it occupied only small amount of sediments (20 L = 87 kg).

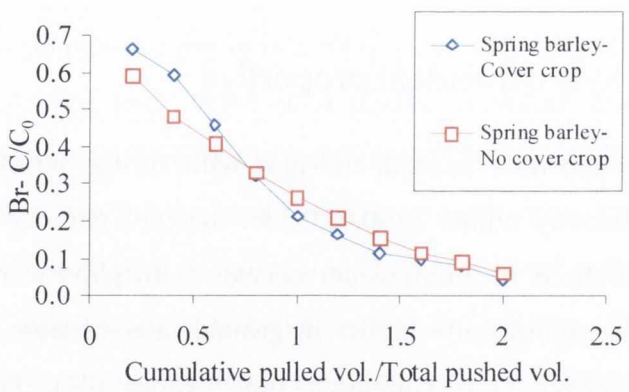


Figure 6.24 Relative concentration profiles of conservative tracer (Br^-) beneath spring barley with cover crop and without cover crop rotations from the 4-h in situ nitrate push-pull test; The term C represents the concentration of the sample pulled from the well. The term C_0 represents the concentration of the solution originally pushed into the well

6.6.6 Variations in denitrification rates

Mean N_2O production rates were similar ($p>0.05$) in shallow groundwater beneath both plots i.e. spring barley with cover crop (mustard) and without cover crop rotations, being respectively 2.27 and 1.97 ng N $kg^{-1}d^{-1}$ (Figure 8.25a). However, in one well beneath each

plot, there was no N_2O production observed. The plot which was cultivated with spring barley and cover crop rotation (mustard) significantly increased N_2 production rates in shallow groundwater (Figure 8.25b) showing the mean value of 7.61 μg N kg^{-1} d^{-1}. Surprisingly, there was no N_2 production in shallow groundwater beneath the plot which was cropped with only spring barley. TDN rates were significantly higher in groundwater beneath the plot which was cropped with spring barley and cover crop rotations ($p<0.05$). The mean TDN rates in two different plots were 7.61 and 0.002 μg kg^{-1} d^{-1}, respectively, in spring barley with cover crop and spring barley without cover crop (Figure 8.26a), which was equivalent to respectively 0.033 and 0.0001 mg N L^{-1} d^{-1}. Groundwater in the wells beneath spring barley with mustard rotation showed 0.07% losses of the injected NO_3^-, whereas the losses in wells beneath spring barley with cover crop were negligible. The N_2O/N_2O+N_2 ratio was approximately 1 in the wells which were located beneath the plot which had no cover crop as there was no N_2 production being measured within this short incubation period (Figure 8.26b). Whereas, the wells beneath the plot cultivated with cover crop and spring barley rotation showed a very negligible ratio 0.001.

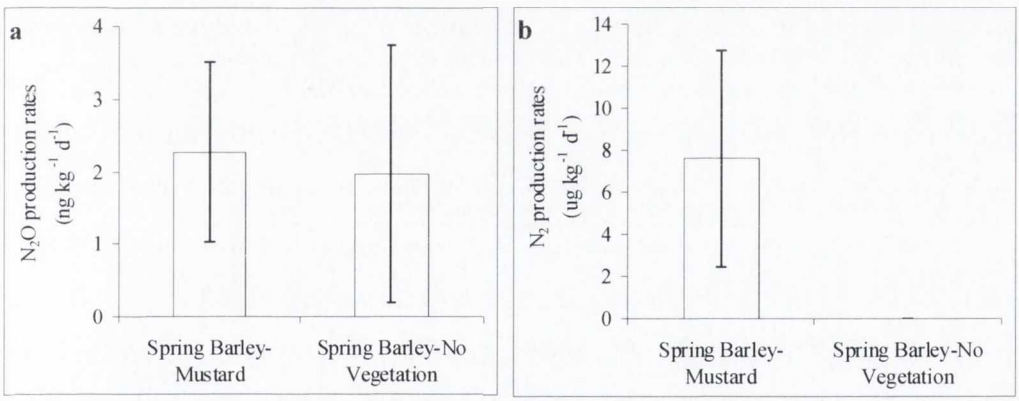


Figure 6.25 N_2O, (a) and N_2, (b) production rates in two different crop rotation systems: spring barley with cover crop rotation and spring barley without cover crop rotation

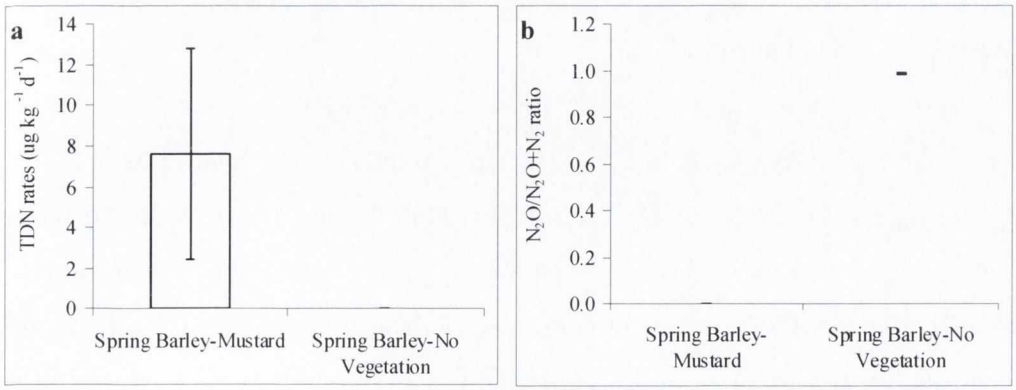


Figure 6.26TDN, (a) and N_2O mole fraction (N_2O/N_2O+N_2), (b) in two different crop rotation systems: spring barley with cover crop rotation and spring barley without cover crop rotation

6.6.7 Interpretation of in situ denitrification rates in differently managed arable land

Shallow groundwater in sand and gravel aquifer has a free draining nature of rainwater percolating through the rooting zone bearing dissolved nutrient such as NO_3^- and DOC. Denitrification in such aquifer generally low due to the absence of energy sources for denitrifiers like organic C and the presence of high DO, because denitrifiers are facultative anaerobes that need anaerobic conditions e.g., DO <2 mg L^{-1} (Böhlke et al., 2007), 2-3 mg L^{-1} (Bates and Spalding, 2005), <4 mg L^{-1} (Böhlke and Denver, 1995). Moreover, high pH can be another reason of low denitrification because pH is close to the maximum pH range for denitrification to occur. Rust (2000) noted that the optimum range of pH for denitrification as 5.3-8.0. However, adoption of cover crop (mustard) with spring barley rotation was appeared to significantly increase denitrification rates in shallow groundwater. This can be attributed to the increased DOC concentrations beneath this field, as cultivation of mustard after harvesting the spring barley significantly increased DOC concentrations in shallow groundwater. However, the DOC concentration in groundwater beneath this cover cropping systems is not sufficient to completely reduce the available nitrate in groundwater, being mean concentration of approximately 15 mg N L^{-1}, because denitrification process was found to reduce approximately 0.033 mg N L^{-1} per day that accounted for only 0.07% of the injected NO_3^-. Nonetheless, the mean denitrification rate in groundwater beneath cover crop with spring barley during the short incubation period implies that denitrification can reduce nitrate contamination while DOC concentration is increased. The mean ambient net nitrate concentrations of 13.6 and 20.2 mg N L^{-1}, respectively in wells beneath the cover crop system and without cover crop plot also supporting the results of short incubation rates of denitrification. Another implication of adopting cover crop with spring barley is that the end product of denitrification is mostly N_2 (approximately 100%), whereas groundwater beneath the plot which had no cover crop showed 98% N_2O over the TDN.

Despite the high DO concentrations in such groundwaters, mustard as a cover crop after harvesting the spring barley increased denitrification rates possibly due to the presence of some microsites which have comparatively more anaerobic environment than the surrounding areas. Presence of clay lenses in such aquifer can possibly create the anaerobic microsites. In this shallow aquifer, existence of clay lenses were observed in subsoil and glacial till (discussed in chapter 3). Premrov (2011) noted the presence of clay band in this

shallow aquifer. Moreover, the occurrence of aerobic denitrification in this aquifer is not surprising as it is reported in literature that aerobic denitrification can occur at even 80% oxygen saturation conditions (Carter et al., 1995). Even though aerobic denitrification (~80% air saturation) can take place in groundwater, denitrification actually seems more likely under locally anaerobic conditions within microsites in particulate organic matter (Hammersley and Howes, 2002), heterogeneous organic rich patches of sediments (Jacinthe et al., 1998) or biofilms (Seiler and Vomberg, 2005). Therefore, it is very clear in such aquifer that denitrification is C limiting in groundwater beneath this arable land. Even with high DO concentration, denitrification occurred in microsites (clay lenses) can reduce nitrate concentrations. Groffman et al. (1996) reported that even low rates of denitrification can consume significant amounts of NO_3^--N. Without additional C supply in groundwater beneath arable land denitrification is very negligible and produce only N_2O without its reduction further to N_2. No NH_4^+ production in this short incubation study was detected, suggesting that shallow groundwater within this depth did not contribute to DNRA or if it did, the produced NH_4^+ might be re-nitrified to nitrate. In addition, produce NH_4^+ can be fixed in the clay lattice or can be converted to N_2 via anammox but in such aerobic environment anammox may not be realistic.

6.6.8 Conclusions

Groundwater denitrification in sand and gravel aquifers with comparatively aerated environments (DO>10 mg L^{-1}) beneath arable land is not reported to be a significant pathway of natural NO_3^- reduction. This was mainly due to the lack of anaerobic conditions and the unavailability of organic C. However, introduction of cover crop e.g., mustard into the agricultural management activities was appeared to enhance the denitrification process mainly by adding more organic C than the plot which has not been cropped with cover crop. The spring barley-cover crop rotation systems reduced 0.033 mg N L^{-1} per day in shallow groundwater (>4 m bgl) in even such well drained (vulnerable) aquifer. In addition, the end product of denitrification process was mainly N_2 (~100%) which is environmentally inert, suggesting that inclusion of cover crop with spring barley can reduce indirect N_2O emissions to the atmosphere. These results suggest that enhanced C concentrations into the sediments and groundwater beneath arable systems can reduce nitrate contamination in ground and surface waters and indirect N_2O emissions to the atmosphere.

CHAPTER 7. ASSESSING GROUNDWATER QUALITY AND DISSOLVED C AND N LOSSES TO THE SURFACE WATERS

7.1 Overview of this chapter

Quality of groundwater within agricultural systems at the sites under study was evaluated with context to common ions and metals to understand if there is any concern for groundwater contamination with specific elements and if there is any elements that can suppress microbial activities that contribute denitrification in groundwater (i.e. at a toxic level to denitrifier harbouring genes). In addition, annual emissions of dissolved C and N via groundwater to the surface waters were assessed based on the hydraulic loadings of each site during 2009 and 2010.

7.2 Quality of groundwater in the study sites

7.2.1 Background

Quality of groundwater with respect to the dissolved mineral and metal contaminations is of local and global concern due to its connection to human health and the environment. Some of the pollutants are influenced by the local lithology but some are not, termed as global. Dissolved mineral constituents can be hazardous to animals or plants in large concentrations; for example, too much sodium in the water may be harmful to people who have heart trouble (USGS, 2011). The parameters may not be an issue of concern if they are under defined limit, the Natural Background Level (NBL). These values are indicative of natural conditions, beyond which it is likely that the groundwater has been polluted to some degree. The NBLs are not environmental standards; they are a means of providing a datum to determine if there are anthropogenic impacts on groundwater quality (QMC, 2007). Some of the metals under the study areas were found to be higher than the NBL. Local soil and bedrock type are very important determinants of groundwater contamination. Free draining soil and subsoils create a vulnerable condition to pass point and non-point pollutants from surface to groundwater and, eventually, to the receptors.

Relationships between the ambient metal concentrations in groundwater and denitrification end products (N_2O and N_2) are not widely reported. Understanding such relationships are interesting to get insights into the impacts of local hydrogeochemistry and mineralogy on the availability of NO_3^- in groundwater and the risk of NO_3^- delivery to surface waters. Denitrification can cause a substantial reduction of NO_3^- while it is passing through the landscapes towards the receptors but it is a function of local hydrogeochemistry. Therefore, the relationships between denitrification and metals in groundwater may help managing NO_3^- contamination in groundwater and its delivery to surface waters.

7.2.2 Methodology

Losses of dissolved C and N via groundwater were estimated based on the concentrations of C and N and the volume of groundwater discharged to the rivers. The estimated volume of groundwater was shown in Chapter 5 and the concentrations of C (DOC, CO_2 and CH_4) and N (TN, DON, NO_2^-, NH_4^+, N_2O, N_2) were shown in Chapter 5 and 6, respectively.

7.2.3 Results

7.2.3.1 Basic cations in groundwater and their relationships with denitrification

Mean Na^+ concentrations over the two years (2009-2010) were 20.8, 23.6, 15.7 and 11.9 mg L^{-1}, respectively, at JC, SH, OP and DG, irrespective of depth (Figure 7.1). It did not differ significantly between sites, but increased significantly ($p<0.001$) with depth at JC and SH sites. Within each site the spatial distribution of Na^+ concentrations were very low (Appendix 10). Na^+ showed negative correlation to N_2O ($r=-0.31$) and positive correlation to N_2 concentrations ($r=0.45$; Table 7.1).

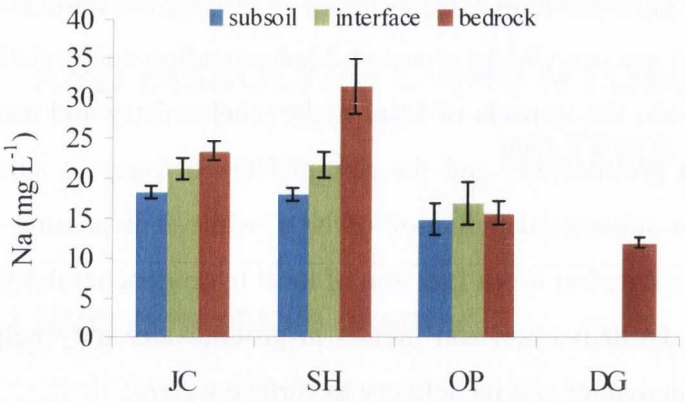


Figure 7.1Mean (±SE over time) Na^+ concentrations in four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

Mean K^+ concentration in all sites and depths were similar ($p>0.05$) with being 3.70, 4.91, 2.18 and 3.85 mg L^{-1} at JC, SH, OP and DG, respectively (Figure 7.2). The K^+ did not show significant correlation to either N_2O or N_2 (Table 7.1). K^+ concentrations were measured higher in autumn than other sampling periods. However, K^+ distribution in each site between different wells is more heterogeneous than Na^+ suggesting biogeochemically more reactive nature of K^+ (Appendix 10). Moreover, its application as organic or inorganic forms can make heterogeneous distribution across the catchment.

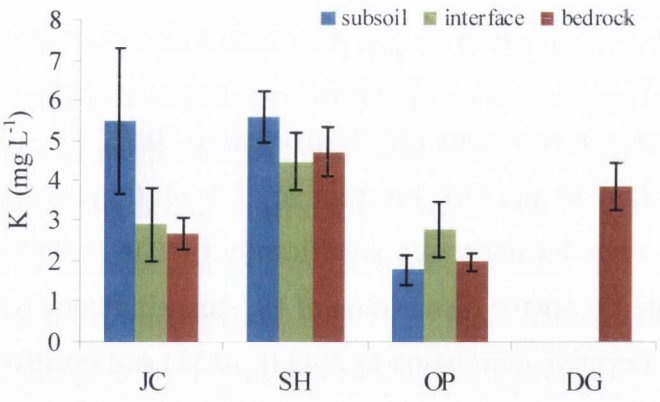


Figure 7.2 Mean (±SE over time) K^+ concentrations at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

The Ca^{2+} concentrations in groundwater under the study areas were comparatively high, but was similar across sites ($p>0.05$), with being site mean values of 85, 83, 82 and 113 mg L^{-1} at JC, SH, OP and DG, respectively (Figure 7.3). Conversely, Ca^{2+} concentrations differed significantly among depths ($p<0.05$). All sites showed that Ca^+ concentrations

were within the range of NBL unlike OP. Spatial distribution at each site was very small (Appendix 10), but temporal changes were moderate with CV values 25, 31, 25 and 18% at JC, SH, OP and DG, respectively. Ca^{2+} showed a significant positive and linear relation (r=0.54) with N_2O (Figure 7.4) but negative correlation (r=-0.19) to N_2 (Table 7.1).

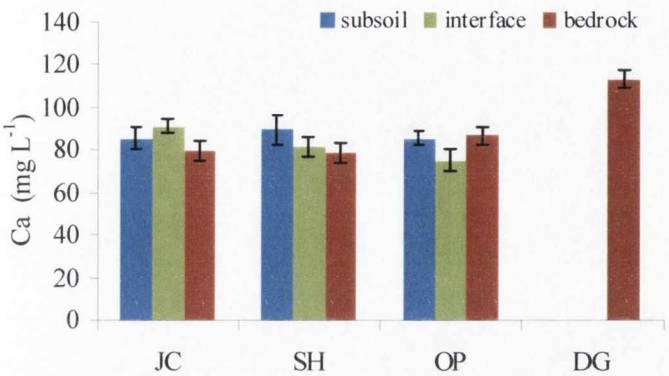


Figure 7.3 Mean (±SE over time) Ca^{2+} concentrations at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

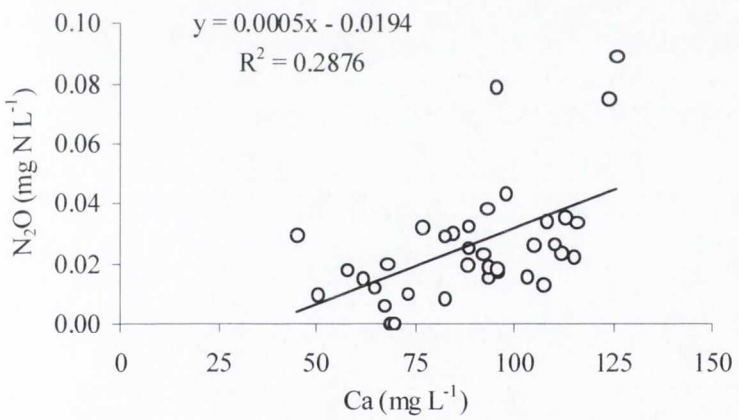


Figure 7.4 Relationship between N_2O concentrations and Ca concentrations in groundwater (n = 36)

The Mg^{2+} concentrations across sites showed different phenomenon than other three basic cations because it was significantly higher ($p<0.05$) at JC and SH sites than at OP and DG sites with the mean values observed were 18.5, 20.8, 13.1 and 9.7 mg L^{-1} at JC, SH, OP and DG, respectively (Figure 7.5). Mg^{2+} showed moderate temporal variabilities in each site being the calculated CV values were 20, 26, 28 and 26% at JC, SH, OP and DG, respectively. Its spatial distribution was also moderate-to-high in each site between wells (Appendix 10). A very interesting connection of Mg was observed with N_2O and N_2 productions in groundwater being observed negative correlation to N_2O concentrations

(Table 7.1) and positive to N_2 concentrations (Figure 7.6). High Mg concentrations in groundwater corresponded with low Eh values (Figure 7.7) which can be the driver of increased N_2 production.

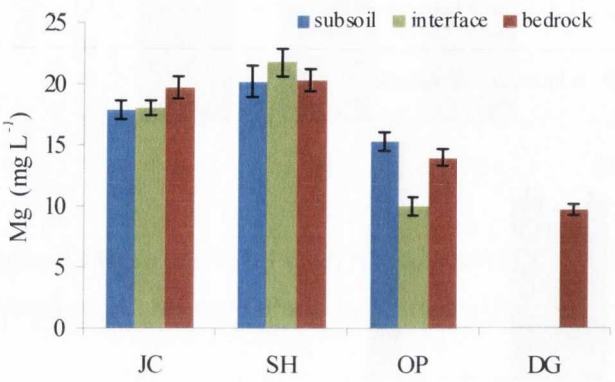


Figure 7.5 Mean (±SE over time) Mg^+ concentrations at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

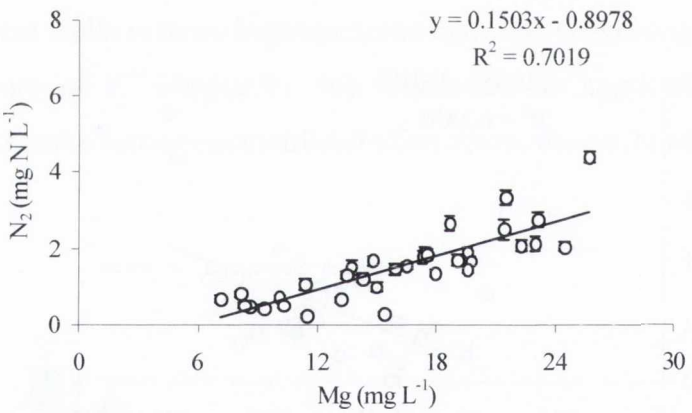


Figure 7.6 Relationships between denitrified N_2 and ambient Mg concentrations in groundwater (n=36)

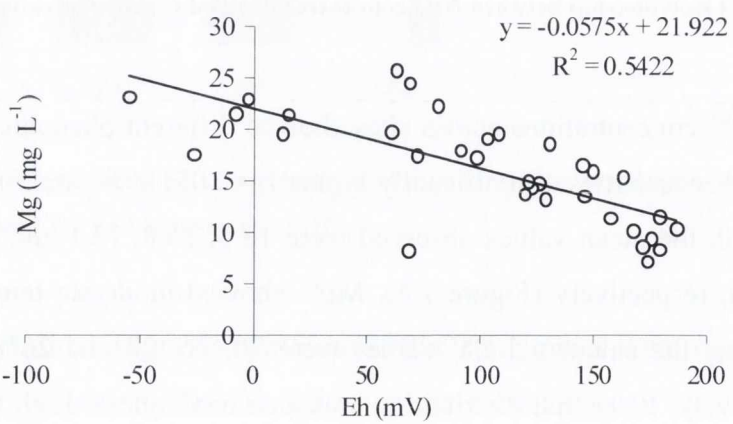


Figure 7.7 Relationships between Mg concentrations and redox potential (Eh) in groundwater (n=36)

The EC values in groundwater under the study sites were comparatively higher with similar amount between sites and depth ($p>0.05$) with being the site mean 490, 529, 479 and 628 $\mu S\ cm^{-1}$ at JC, SH, OP and DG, respectively (Figure 7.8). EC in groundwater increases N_2O accumulation (Figure 10.9) and decreases N_2 production. A very strong correlation between EC and Ca concentrations indicated that EC is mainly controlled in groundwater by Ca^{2+} (Figure 7.10) which is not surprising.

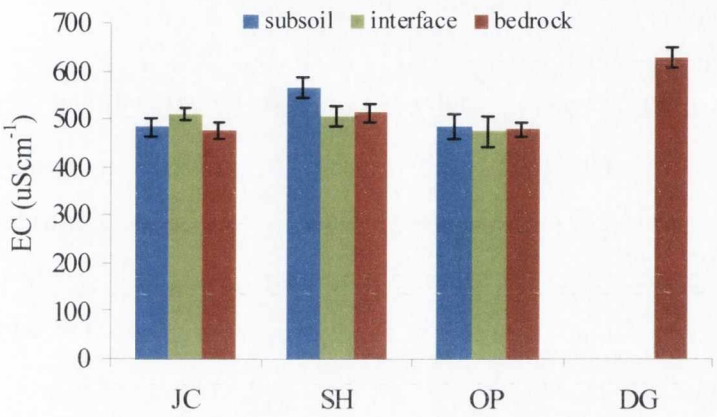


Figure 7.8 Mean (±SE over time) EC at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

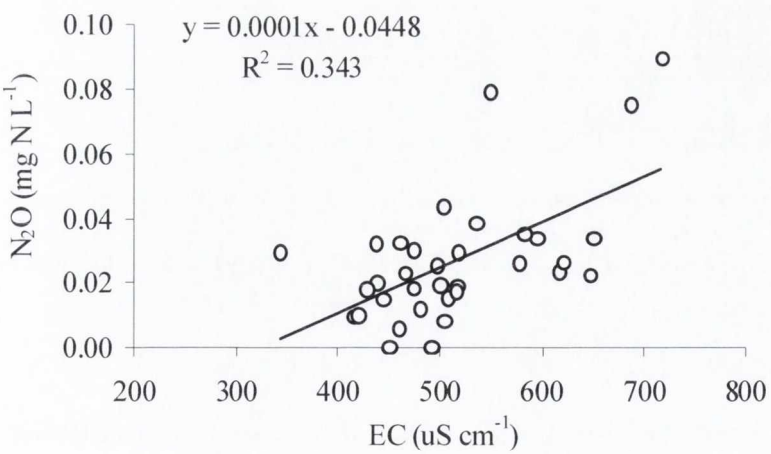


Figure 7.9 Relationships between denitrified N_2O concentrations and electrical conductivity (EC) in groundwater (n = 36)

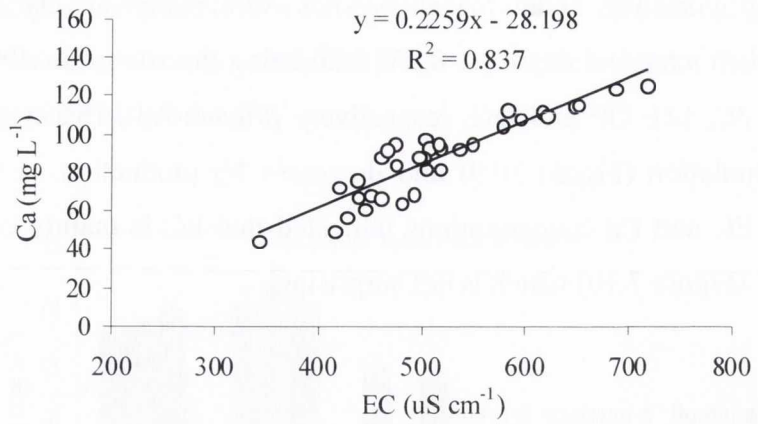


Figure 7.10 Relationships between Ca concentrations and EC (µS cm^{-}) in groundwater (n = 36)

Chloride (Cl^-) can be used as a conservative tracer in groundwater to understand NO_3^- attenuation processes e.g., physical and biogeochemical. Mean Cl^- concentrations (Figure 7.11) were significantly higher at JC (33 mg L^{-1}) than at SH (22 mg L^{-1}), OP (17 mg L^{-1}) and DG (19 mg L^{-1}). Highest Cl^- concentrations were observed in bedrock with lowest in subsoil unlike the SH site.

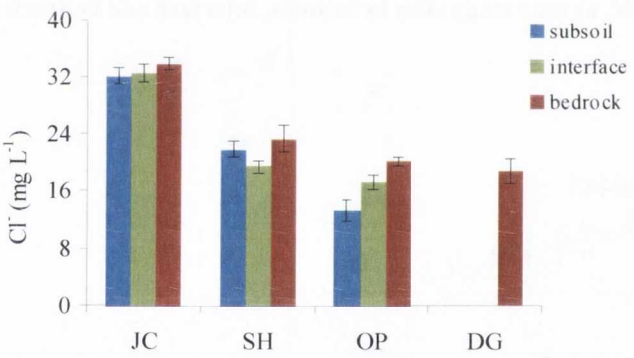


Figure 7.11Mean (±SE over time) Cl^- concentrations at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

7.2.3.2 Metals in groundwater and their relationships with denitrification

Mean Zn concentrations at JC, SH, OP and DG were 2.15, 2.26, 1.23 and 3.10, respectively which were similar ($p>0.05$) across sites and depths (Figure 7.12). Zn showed larger temporal changes at all sites with the CV ranged from 240-242%. The Zn concentrations in groundwater at its present range did not show significant relations either with N_2O or N_2 (Table 7.1).

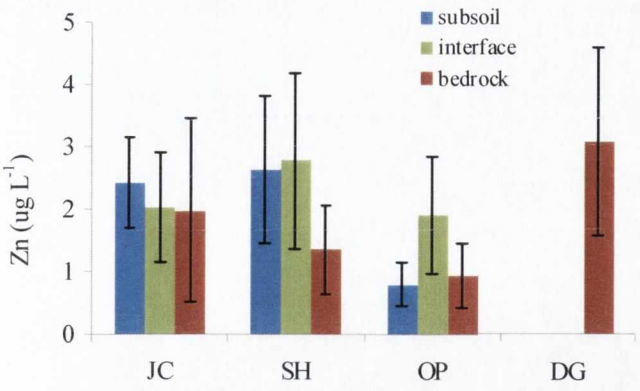


Figure 7.12 Mean (±SE over time) Zn concentrations at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

Mean Cu concentrations were significantly higher ($p<0.05$) at JC (3.87 μg L^{-1}) and SH (3.03 μg L^{-1}) sites than at OP (2.04 μg L^{-1}) and DG (2.25 μg L^{-1}) sites (Figure 7.13). Cu distributions between wells within each site showed moderate spatial variability (Appendices 10). Temporal variability of Cu concentrations was large showing the mean CV values 143, 100, 108, 157%, respectively at JC, SH, OP and DG. Cu concentrations showed significant positive correlation with N_2 ($p<0.05$), whereas it showed very weak negative correlation with N_2O ($p>0.05$; Table 7.1).

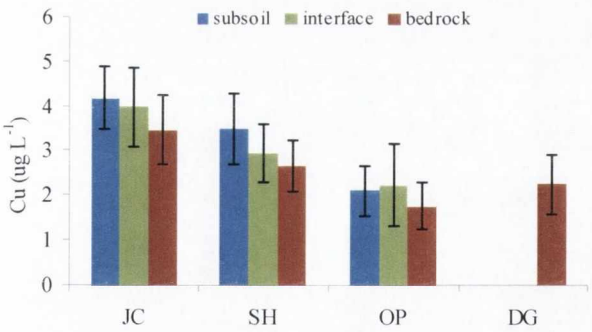


Figure 7.13 Mean (±SE over time) Cu concentrations at four study sites in subsoils, interface and bedrock in JC, SH and OP and in bedrock in DG

The Cd concentrations in all sites were similar ($p>0.05$) showing mean values 0.65, 0.61, 0.88 and 0.70 μg L^{-1} at JC, SH, OP and DG, respectively (Figure 7.14), but it differed significantly among depths ($p<0.05$). It showed a very high temporal variability (CV: 163, 147, 157 and 189%, respectively) but spatial variability was comparatively low (Appendix 10). With the range of present concentrations no negative effect of Cd on microbial

denitrifiers was noted as it showed a very week positive correlation with denitrification end products (Table 7.1).

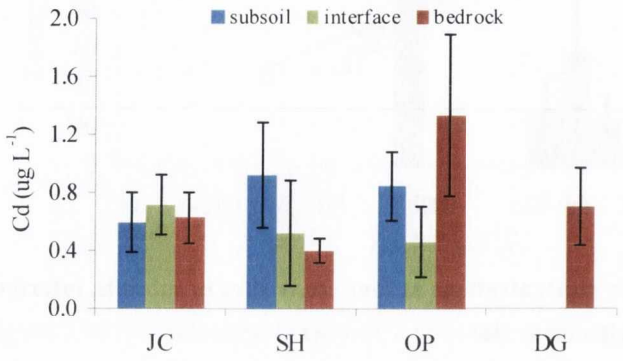


Figure 7.14 Mean (±SE over time) Cd concentrations at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

The Cr concentrations were significantly higher at OP (4.03 μg L^{-1}) than JC (2.09 μg L^{-1}), SH (1.56 μg L^{-1}), and DG (2.46 μg L^{-1}) (Figure 7.15). Cr concentrations across sites were higher than the NBL value 2 μg L^{-1}. However, its temporal variability (CV 101, 102, 112 and 103%, respectively) was larger than its spatial variability (Appendix 10). Cr was positively correlated to N_2O concentrations but negatively to N_2 (Table 7.1).

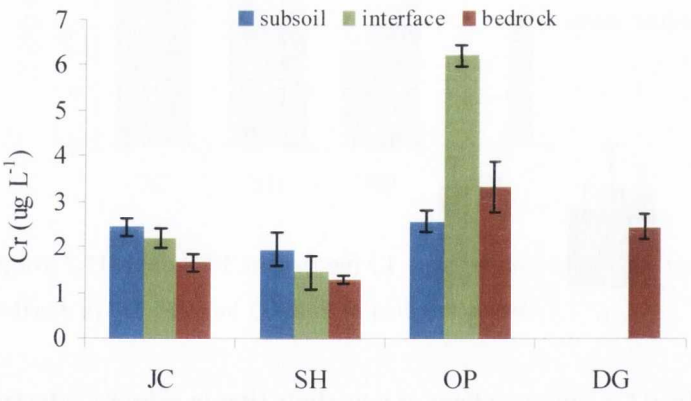


Figure 7.15 Mean (±SE over time) Cr concentrations at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

The Ni concentrations were similar in all sites averaging 3.26, 2.03, 3.70 and 3.34 μg L^{-1} at JC, SH, OP and DG, respectively (Figure 7.16). Within the range of this concentration Ni did not show any inhibitory effects on denitrification as it was positively but not significantly correlated to denitrification end products (Table 7.1). Like other heavy metals Ni showed higher temporal variability than spatial.

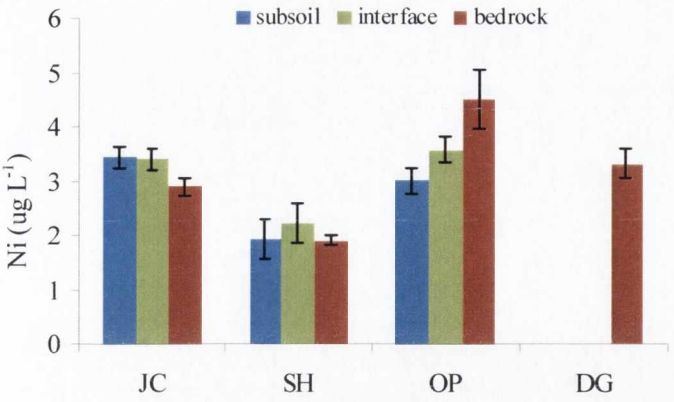


Figure 7.16 Mean (±SE over time) Ni concentrations at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

Mean Pb concentrations across sites being measured monthly over the two years periods (Feb, 2009- Jan, 2010) ranged from 4.52 μg L^{-1} at OP to 7.29 μg L^{-1} at SH. However, like other heavy metals, Pb concentrations were similar ($p>0.05$) across sites as well as depths (Figure 7.17). Pb concentrations showed a very week positive correlation to the denitrification end products (Table 7.1). Groundwater quality in connection with the major ions and metals were evaluated and summarised in Table 7.2.

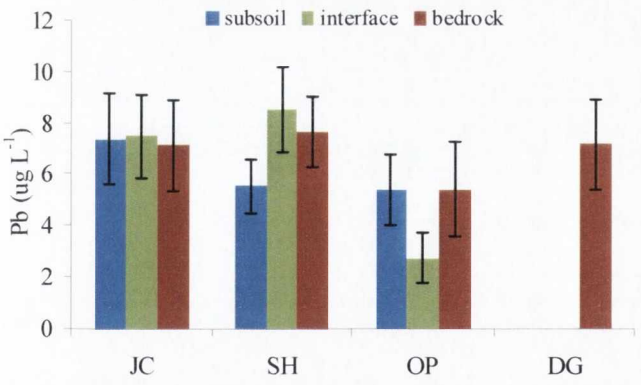


Figure 7.17 Mean (±SE over time) Pb concentrations at four study sites in subsoils, interface and bedrock at JC, SH and OP and in bedrock at DG

Table 7.1Spearman Rank Order correlation coefficient (r) between N_2O, N_2 and groundwater metal ions

	N_2O	N_2	Na	K	Ca	Mg	Cu	Zn	Cd	Cr	Ni	Pb
N_2O												
N_2	-0.54*											
Na	-0.31*	0.45*										
K	0.14ns	-0.05 ns	0.00 ns									
Ca	0.54**	-0.19 ns	-0.50**	0.43*								
Mg	-0.47**	0.84**	0.59**	-0.22*	-0.59**							
Cu	-0.10 ns	0.27*	0.20 ns	0.27*	0.12 ns	0.07 ns						
Zn	-0.08 ns	0.16 ns	0.32*	0.01 ns	-0.25*	0.27*	0.11 ns					
Cd	0.18 ns	0.16 ns	0.02 ns	0.14 ns	0.11 ns	-0.05 ns	0.50**	0.83**				
Cr	0.30*	-0.06 ns	-0.04 ns	0.04 ns	0.13 ns	-0.23*	0.26*	0.57**	0.72**			
Ni	0.19 ns	0.13 ns	-0.10 ns	-0.16 ns	0.12 ns	-0.51**	0.12 ns	0.04 ns	0.37*	0.54**		
Pb	0.05 ns	0.20 ns	0.07 ns	0.23*	0.13 ns	0.17 ns	0.43*	0.19 ns	0.27*	0.13 ns	-0.02 ns	

ns non-significant; * significant at 5% level; and ** significant at 1% level of probability

Table 7.2 Evaluation of groundwater quality with respect to the water quality standards in the study sites

Elements	WQS*	EQS**	Measured Concentrations				Pollution Status			
Constituents			JC	SH	OP	DG	JC	SH	OP	DG
NO_3^- (mg N L^{-1})	11.3	8.47	3.7	0.7	11.0	14.6			†‡	†‡
NO_2^- (mg N L^{-1})	0.2	0.11	0.01	0.00	0.20	0.00			†‡	
NH_4^+ (mg N L^{-1})	0.12	0.065	0.13	0.02	0.04	0.01	†‡			
Cl^- (mg N L^{-1})	250	187.5	22.2	15.5	25.6	20.0				
SO_4^{2-} (mg N L^{-1})	250	187.5	32.8	21.6	17.0	18.9				
Na (mg L^{-1})	200	150	20.8	23.6	15.7	11.9				
K (mg L^{-1})	12	12	3.7	4.9	2.2	3.8				
Ca (mg L^{-1})	200	200	85	83	82	113				
Mg (mg L^{-1})	125	50	18.5	20.8	13.1	9.7				
EC (µS cm^{-1})		1875	490	529	479	628				
Fe (µg L^{-1})	300	200	480	289	89	45	†‡	†		
Mn (µg L^{-1})	50	50	301	130	9.05	5.05	†‡	†‡		
Cu (µg L^{-1})	1000	1500	3.87	3.03	2.04	2.25				
Zn (µg L^{-1})	5000	8	2.15	2.26	1.23	3.10				
Cd (µg L^{-1})	10	3.75	0.65	0.61	0.88	0.70				
Cr (µg L^{-1})	50	37.5	2.09	1.56	4.03	2.46				
Ni (µg L^{-1})	20	15	3.26	2.03	3.70	3.34				
Pb (µg L^{-1})	50	18.75	7.29	7.21	4.52	7.15				

*Sources: WQS, water quality standard, Freeze and Cherry, 1979, pp. 385; ** Environmental Quality Standard, sources: Craig and Daly, 2010; † showed the sites which have contaminant concentrations more than the EQS value; ‡ showed the sites which have contaminant concentrations more than the quality standards

7.2.4 Discussion

7.2.4.1 Contamination of groundwater with dissolved common minerals

Mean Na^+ concentrations at JC and SH were higher than the NBL (9-19 mg L^{-1}) suggesting the possibility of groundwater contamination with Na. Furthermore, it is much lower than the EQS (Environmental Quality Standard) value of 150 mg L^{-1} (Craig and Daly, 2010). However, it is not a harmful constituent but it is an indicator of impact on groundwater quality (GSI, 1999). K^+ across sites was lower than the IGV (Interim Guideline Value) 5 mg L^{-1}, showing no concern of groundwater K^+ contamination. These results were comparable with the mean K^+ concentration report of the GSI survey in the principal springs in Ireland of 2.9 mg L^{-1} (Daly et al., 1989). According to the NBL for Mg (QMC, 2007), there was no concern for groundwater contamination with Mg in the present monitoring areas because its concentrations are lower than the NBL. The EC values at DG site are very similar to the EC in the river Funshion (354-630 µS cm^{-1}) nearby the

catchment as reported by Bartley and Johnston (2006). Groundwater EC showed that it is within the NBL values recommended by QMC (2007). Variation in EC concentration is directly related to variation in ion concentration: the greater the number of ions, the higher the value (Dojlido and Best, 1993). A fluctuating pattern in Cl^-/NO_3^- ratios in groundwater suggested that together with physical attenuation processes denitrification or other biogeochemical pathways were driving the abundances of NO_3^- (Chapter 5). Higher Cl^- concentrations in some sites than others were associated with agricultural activities like dirty water irrigation or organic wastes application Richards (1999) and from potassium fertilizer application (Kiely, 1997). The GSI trigger value for Cl^- is 30 mg L^{-1} which is also the EPA IGV value for assessing groundwater quality (EPA, 2003). Nevertheless, Cl^- concentrations across sites were higher than the median value of NBL (18 mg L^{-1}).

7.2.4.2 Groundwater contamination with metals

The Zn contamination across sites was measured lower than the NBL value of 8 µg L^{-1}. However, some of Irish groundwater samples as measured by McGarrigle (2010) were higher than the EQS. Zn concentrations did not show considerable relations either N_2O or N_2, may be due to its low concentrations in groundwater because previous research reports showed no effects of Zn on denitrification in wetland sediments at low concentrations (100 mg Zn kg $soil^{-1}$) but negative influence at high concentrations (500-1000 mg kg $soil^{-1}$) (Holtan-Hartwig et al., 2002; Sakadevan et al., 1999; Vásquez-Murrieta et al., 2006). Cupper contamination with respect to the NBL value (2.5 µg L^{-1}) can be a concern at JC and SH sites. Even though not significant, Cu showed positive correlation with denitrification (Table 10.1), which was in agreement with past research results of Sakadevan et al. (1999), but was in contrast to Holtan-Hartwig et al. (2002), Vásquez-Murrieta et al. (2006). Occurrence of the highest mean Cu concentration at JC site could be due to the dirty water irrigation practices there in one field (JC2A, JC2B and JC2C), which showed site highest concentration and overall highest concentration as well. The Cd showed a very weak positive correlation with denitrification which is contrasting to previous findings may be due the low concentrations in these groundwaters as it is reported to be inhibitory when present in soil at high concentrations (Bollag and Barabasz, 1979; Holtan-Hartwig et al., 2002). The Cr showed positive correlation with N_2O but negative to N_2, suggesting that Cr may possibly enhance NO_3^- reductase activities but inhibit nitrous oxide reductase activities. Groundwater contamination with Ni showed that it was more

than double of the NBL value (1.7 μg L^{-1}) as recommended by QMC (2007). The Pb concentrations in groundwater showed a weak positive correlation with denitrification even though in literature it is reported to be inhibitory to denitrification at its high concentrations (Bollag and Barabasz, 1979; Vásquez-Murrieta et al., 2006). The Pb concentrations in Irish groundwater are generally lower than the EQS (McGarrigle et al., 2010).

7.2.5 Conclusions

Nitrate concentrations in groundwater exceeded the internationally imposed (50 mg NO_3^- L^{-1} or 11.3 mg N L^{-1}; WHO, 2004) quality criteria in OP and DG sites unlike the JC and SH sites. There was no record of NH_4^+ concentrations above the EQS level in these areas. Only OP site seems to exceed the EQS value for NO_2^-. The present heavy metal status shows no risk of pollution (all are less than the EQS) but considering the NBL levels there is a risk of high heavy metal contamination in these areas especially for Cr, Cu and Ni, being above the NBL values. With regards to denitrification, higher EC values are consistent with higher N_2O emissions such as at DG, in particular. The Na, Mg, Cu, Cd, Ni and Pb have positive impacts on the N_2 production in groundwater unlike K, Zn and Cr. However, considering the N_2O emissions, K, Ca, Cd, Cr and Ni are consistent with the higher N_2O production in groundwater unlike Na, Mg, Cu, Zn. In general heavy metals are toxic to denitrifiers but in these groundwater sites at their current contamination levels with heavy metals no significant inhibitory effects of on denitrification was noted. As a whole, the chemical characteristics of groundwater are acceptable for most uses except for NO_3^- at OP and DG sites.

7.3 Contaminant mass fluxes in the study sites

7.3.1 Introduction

The ability to measure groundwater contaminant flux is increasingly being recognised as crucial in order to prioritise contaminated site cleaning, estimate the efficiency of remediation technologies, measure rates of natural attenuation, and apply proper source terms to model groundwater contaminant transport (Goltz et al., 2007). The excessive amounts of reactive forms of N arisen from agricultural systems are of great ecological and environmental concern. Nitrate is one of the most important forms of reactive N which

migrates from its sources to groundwater and eventually contaminate drinking water and other potential receptors e.g., rivers, estuaries and lakes. The risk of NO_3^- contamination and its likely effects can be measured to take remediation measures by quantifying the mass flux of NO_3^-. Mass flux is a measure of the rate at which contaminant mass is transported, in units of mass per time per area of aquifer orthogonal to the direction of groundwater flow. Einarson and Mackay (2001) argued that contaminant mass flux is more relevant as an indicator of risk at a down gradient water supply well than contaminant concentration in the plume and would be more useful in helping regulators and remediation decision makers. Contaminant mass flux measurement has been the subject of considerable research in the past five years, as scientists, regulators and hazardous waste site managers have begun to realize the importance of measuring contaminant flux, as opposed to traditional measurements of contaminant concentration (SERDP/ESTCP, 2001). Contaminant mass flux can be a function of aquifer permeability. Goltz et al. (2007) stated that contaminant source zone may have the majority of contaminant mass located within low permeability regions. In this case, even though contaminant mass and dissolved concentrations may be large, the flux of contaminant leaving the source zone will be low. Conversely, a smaller source zone in a high permeability region may results in significant contaminant mass flux leaving the area. Various authors applied the mass flux measurement approach to evaluate natural attenuation and quantify natural attenuation rate constants (Bockelmann et al., 2001; Bockelmann et al., 2003; Peter et al., 2004). The objectives of this chapter were to (i) quantify the amount of NO_3^- discharges to the receptors; (ii) quantify the amount of NO_3^- depleted by natural processes while passing through and from the sediments.

7.3.2 Methodology of NO_3^- mass flux estimation

7.3.2.1 Mass flux measurement approach

Contaminant mass fluxes at the field-scale are usually determined at one or more conceptual control planes running perpendicular to the groundwater flow direction. Several measurement and interpretation methods are available which can be grouped into point-scale and integral approaches. The point-scale approach requires data from a multilevel monitoring network (Bockelmann et al., 2003; Einarson et al., 2000), but the integral approach uses data from a monitoring campaign that has to be conducted at one or more monitoring wells (Bockelmann et al., 2001). Multilevel groundwater monitoring wells can

be used in measuring field-scale contaminant mass fluxes (API, 2003). However, the most common and widely used method is flux or discharge measurement based on point measurements at multilevel wells. The common method for quantifying mass flux in groundwater is to sample a control plane at a number of multilevel wells each equipped with a number of vertical sampling points (Kübert and Finkel, 2006) (Figure 7.18). In order to estimate the mass discharge W (g d^{-1}) across a certain area A (m^2) perpendicular to the flow direction the following basic equation was used:

$W = C*q*A$ (Eqn. 7.1)

where C (g L^{-1}) is the contaminant concentration in groundwater, q is the specific groundwater discharge (m d^{-1}) and A is the cross section area (m^2) which is obtained from the product of depth of saturated zone (m) in a control plane and a unit width of control plane (1 m). The discharge q can be estimated as the product of hydraulic conductivity K_{sat} (m d^{-1}) and hydraulic gradient i. Multilevel piezometers were installed at 3 different agricultural catchments in Southeastern Ireland to target groundwater in subsoil (5 m below ground level), at interface (12 m bgl) and in bedrock (20-30 m bgl). Each depth of groundwater e.g., subsoil, interface and bedrock represented an individual control plane. Average values of the entire control plane were used to calculate the mass discharge W_{cp} (Kübert and Finkel, 2006) and total discharge across three control planes per unit width (1 m) of land were estimated by summing up of the average values of three control planes. The efficiency of nitrate attenuation at each control plane was estimated by using the formula described by Dhondt et al. (2006) as below:

$$\text{Efficiency (\%)} = \frac{N_{IN} - N_{OUT}}{N_{IN}} * 100 \quad (Eqn.\ 7.2)$$

where N_{IN} is the nitrate mass flux in the up-gradient and N_{OUT} is the nitrate mass flux in the down-gradient of land.

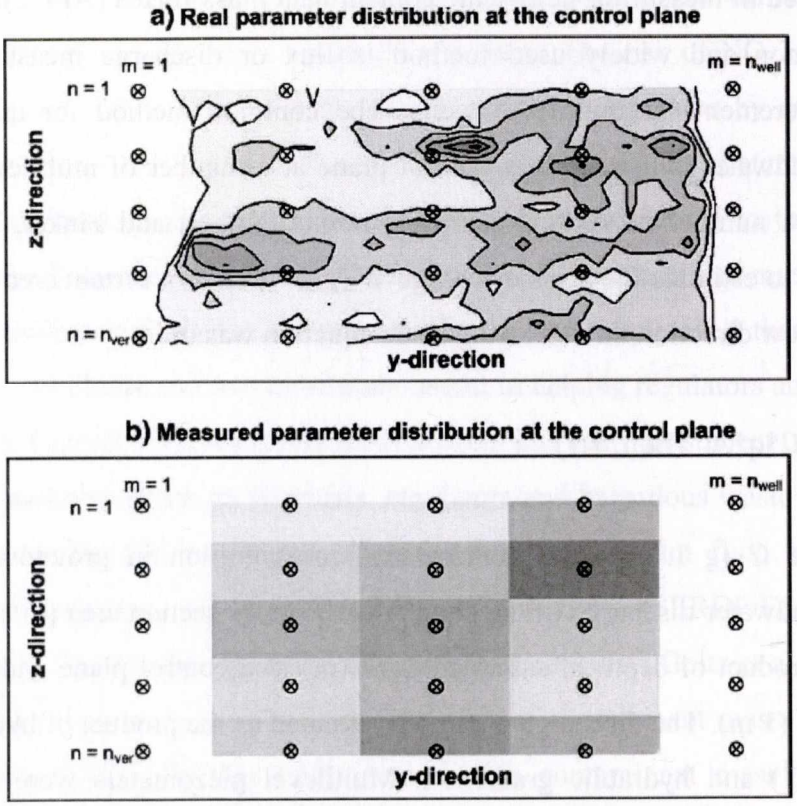


Figure 7.18 Illustration of mass discharge estimation based on the point-scale approach. Top: real mass flux distribution on a control plane. Bottom: areas $A_{n,m}$ associated to each measuring point (Thiessen-polygons) with mass flux distribution from extrapolation (after Kübert and Finkel, 2006).

7.3.2.2 Statistical analysis

The differences in nitrate mass fluxes and Darcian velocities between sites and depths were analysed using a mixed model with site and depths as fixed factors and replication as random factor. As data were approximately log-normally distributed, log transformation was made before analysis. Differences between depths in each site was analysed by Kruskal Wallis-H test.

7.3.3 Results of NO_3^- mass fluxes

Nitrate mass fluxes were significantly different between sites ($p<0.001$). There were no significant differences between the control planes at each site ($p>0.05$) (Table 7.3). Total mass fluxes across all control planes were 0.20, 0.01, 0.48 and 2.92 g d^{-1} (width of the

control plane is 1 m), respectively at JC, SH, OP and DG. Mean Darcian velocity was significantly different between sites ($p<0.001$) showing the highest velocity at DG site and the lowest at SH site (Table 7.3). The Darcian velocities were similar across depth of groundwater ($p>0.05$) even though a consistently increased velocity with increased depths was observed at OP site. The efficiency of natural NO_3^- attenuation at JC site increased with depths of groundwater which ranged from 65% in subsoil plane to 98% in bedrock plane (Table 7.3). At SH, natural attenuation was similar across depths (98%). At the OP site no natural attenuation was observed in subsoil and at interface but in bedrock natural attenuation of NO_3^- was 46% (Table 7.3). Natural attenuation at the DG site was observed as 60% but increased from top to down gradients. Natural attenuation includes attenuation of NO_3^- by physical processes e.g., dilution, dispersion, diffusion and biochemical processes like denitrification, DNRA, anammox, assimilations by microbes and off take by plant roots. Root off take is considered as zero because groundwater depth is greater than 5 m bgl where grass or crop roots are absent. The end products of the biochemical processes (N_2O and N_2) were measured in all wells at all sites over the two years from Feb, 2009 to Jan, 2011 and reported in Chapter 6. Therefore, we had the opportunity to estimate the physical processes of attenuation by subtracting the biochemical processes of NO_3^- reduction along groundwater flow paths. The physical processes of NO_3^- reduction caused 26 to 46% reduction at JC site (Table 7.3). At SH site it caused 20 to 24% reduction of NO_3^-. At OP site in subsoil and at interface no physical attenuation was occurred rather both control planes achieved NO_3^- while passing from top gradient to down gradient (Table 10.3) but in bedrock 35% nitrate was reduced while passing through to the down gradient of the land. At DG natural attenuation was mainly physical processes ca. 56% out of 60%.

Table 7.3Mean contaminant mass fluxes at four sites and three depths of groundwater zones (as of Jan, 2010)

Locations/ Control plane	Depth below water table (m)	Nitrate conc. (mg N L^{-1})	Nitrate mass flux (g N d^{-1})	Darcian velocity (m d^{-1})*	Total natural attenuation (%)	Biochemical attenuation (%)	Physical attenuation (%)
JC							
Subsoil	4.5±0.5	5.16±0.98	0.020±0.001	0.0002±0.0001	65±5	33±2	32±2
Interface	10.0±0.0	4.13±0.23	0.007±0.002	0.0006±0.0005	76±8	50±2	26±2
Bedrock	18.5±0.5	3.34±0.11	0.157±0.015	0.0004±0.0002	98±1	54±1	46±2
SH							
Subsoil	4.0±0.0	0.48±0.05	0.002±0.000	0.0001±0.0001	97±0	74±2	23±2
Interface	7.0±0.0	0.56±0.19	0.001±0.000	0.0001±0.0000	98±0	78±2	20±2
Bedrock	17.0±0.0	0.49±0.05	0.002±0.000	0.0001±0.0000	98±0	74±4	24±3
OP							
Subsoil	4.0±0.0	9.11±0.08	0.006±0.001	0.0003±0.0000	-14±1	8±1	-6±1
Interface	7.0±0.0	11.98±0.13	0.069±0.002	0.0004±0.0002	-103±10	7±1	-96±1
Bedrock	23.0±0.0	12.29±0.22	0.379±0.024	0.0007±0.0001	46±5	9±1	37±1
DG							
Bedrock	17.0±1.0	16.22±0.23	2.732±0.184	0.0023±0.0006	60±10	4±1	56±1

*mean ± SE

7.3.4 Discussion

Mass flux measurement using the above method greatly depends on the estimated hydraulic conductivity values and hydraulic gradients which are spatially and temporally variable. Measurements of these parameters are not always very absolute which results in an uncertainty in measuring the mass flux. However, the used method can give an approximation of field-scale mass flux. At the JC site Fenton et al. (2009a) estimated NO_3^- mass fluxes in 4.2 ha land on the top gradient of the JC farm (48 ha) at three control planes by transect method. From their study with 17 shallow wells (1.5 to 4.3 m bgl), the estimated NO_3^- mass fluxes were highest in the top plane (top gradient) and lowest in the compliance plane (down gradient), indicating the presence of natural attenuation. The authors also estimated 42% contaminant mass flux reduction efficiency from the top control plane to middle plane and a 64% from the middle plane to the compliance plane. Their NO_3^- mass flux reduction efficiency is comparable to our results as we observed a mean NO_3^- mass flux reduction efficiency of 65% in our subsoil control plane (Table 7.3) which was slightly deeper (5 to 6 m bgl) than their depth (1.5 to 4.3 m bgl). Nitrate mass flux reduction efficiency was increased with the depths of control plane in all sites which might be attributed to the reduction of NO_3^- while passing vertically from subsoil to interface and from interface to bedrock as well as horizontally from top gradient to down gradient of the field. The reduction in the flux along the flow path is a good estimate of natural attenuation of the plume as a whole (API, 2003). Nitrate mass fluxes across sites are comparable to the hydrogeochemical conditions of the sites as a higher permeability of water was observed at OP and DG sites than JC and SH sites (Chapter 5). Areas with higher permeability transport greater NO_3^- fluxes to ground and surface waters (Fenton et al., 2009a). In addition, biogeochemical NO_3^- reduction was significantly lower at OP and DG sites than at JC and SH sites (Table 7.3). A remarkable feature of NO_3^- attenuation observed across sites was that at JC and SH sites biogeochemical attenuation mainly denitrification was higher than physical attenuation which was far opposite at OP and DG sites. Nitrate attenuation across the top to down gradient of land is not homogeneous, rather, it follows a heterogeneous pattern may be due to the heterogeneity of groundwater physical and chemical conditions and variability in N management e.g., application in different rates and types in different plots. Similar pattern of heterogeneity of NO_3^- attenuation was reported by Fenton et al. (2009a) in shallow wells at JC. At the DG, Landig (2009) measured nitrate mass flux by a porous media assumption in three different control planes (680, 800 and 385 m width) and reported that total nitrate mass flux was

1850 kg yr^{-1}, in high hydraulic conductivity (2.9 x 10^{-6} m s^{-1}) conditions. The author's result is equivalent to 2.62 g d^{-1}, if we convert the width to a unit width (1 m), which is very well comparable to our estimation (2.92 g d^{-1}). The mass flux and attenuation efficiency values at four sites are quite contrasting to each other and seem to be consistent to nitrate concentrations in the down gradient wells and the existing hydrogeological conditions. At the SH farm, NO_3^- concentration in the wells near the stream is very low where the NO_3^- mass flux/discharge was lowest (Table 7.3). This site seems to be a promising area of mitigating NO_3^- contamination because of its highest NO_3^- attenuation capacity (mainly biochemical attenuation) across sites (Table 7.3). AT OP, natural attenuation is low in subsoil and interface resulting in a higher accumulation of NO_3^- may be due to the presence of clay lenses/inter bedded clay in these control planes (borehole log-Appendices 1-4). In bedrock zone there is a considerable percentage of attenuation but the mean NO_3^- concentration is higher than the upper planes which can be happened due to NO_3^- delivery by some other pathway as the bedrock is gray limestones with comparatively higher permeability (Table 7.3) or a re-nitrification of ammonium (produced in microsites or clay band at upper planes and leached down to bedrock) can increase NO_3^- concentration. This NO_3^- attenuation across sites is again comparable with the measured denitrification rates across sites (Chapter 6).

7.3.5 Conclusions

Quantification of field-scale NO_3^- mass flux and discharge to the receptors is of great ecological and environmental importance to estimate the rates of field-scale NO_3^- attenuation and to evaluate the efficacy of remediation technologies. This will also help farm managers and farmers to plan location/plot specific N input management to protect ground and surface water NO_3^- contamination. Nitrate mass flux in the porous media from the top gradient to the down gradient in the field is heterogeneous due to the heterogeneous nature of the hydrogeochemical properties in groundwater. However, the amount of natural attenuation generally increases with increase in the length of field from the top gradient to down gradient resulting in a higher attenuation near the stream/receptors. Natural attenuation increases with the increase in the depth of control plane showing the lowest in the top control plane (subsoil) and highest in the bottom control plane (bedrock). These phenomena are suggesting that NO_3^- attenuation occurs while groundwaters flow towards both vertical and horizontal directions. Estimation of NO_3^- flux across the boundary to a receptor is an important estimate of loading to the receptors. Contaminant mass flux should

be estimated across more than one control plane to monitor natural attenuation. This allows the identification of hot spot areas where intervention other than natural attenuation may be needed to protect receptors. Physical attenuation is higher in free drained sandy aquifers than the poorly drained clayey aquifers that in turn have higher biochemical attenuation.

7.4 Estimation of dissolved C and N delivery from groundwater to the surface waters

7.4.1 Estimation approach

Carbon balance estimates in Europe and Irish grasslands have high uncertainty, causes of which are not well understood (Cernusca et al., 2008). Siemens (2003) argued that the gap between atmospheric and land based estimates of C budgets in Europe could be the result of DC leaching, which decouples the direct exchange of C between atmosphere and biosphere. Soluble C transport from terrestrial ecosystems accounts for a substantial component of the global (Kessler and Harvey, 2001) and European (Kindler et al., 2011; Ciais et al., 2008; Siemens et al., 2003) C balance. The requirement of the river transport of C from land to ocean has recently been highlighted by Ciais et al. (2008). Dissolved C (DC) and Dissolved N (DN) losses from both grass and crop based agricultural systems were conducted because these parameters were expected to be the causes of high uncertainty in ecosystem level C and N balance. In comparison to forest ecosystems, very little information on DC and DON losses in grassland and croplands is available (van Kessel et al., 2009). Hydrology (e.g., precipitation and discharge) is an important control of N loss from watershed (Zhu et al., 2011). Dissolved C and N feeding from groundwater to surface waters were estimated using the water balance approach (Misstear et al., 2008; Misstear et al., 2009), with the assumptions that-(i) dissolved N_2O and CO_2 concentrations over time are balanced steadily by simultaneous production and reduction processes; (ii) the effective rainfall (ER) equals to the amount of water that reaches the potentiometric surface as overland and lateral flows in these fields are assumed to be zero (Fitzsimons and Misstear, 2006) (iii) volume of water delivered from groundwater to the surface waters is approximately equal to the volume of water entered the groundwater system i.e., the ER, because the GWT at the beginning (1st October) and at the end (30th September) of a hydrological year remains approximately same; and the change in groundwater storage is almost zero (Fitzsimons and Misstear, 2006). These assumptions are supported by the

postulation by Fitzsimons and Misstear, (2006) that 'over a long period, the aquifer outflow (discharge) should be equivalent to the inflow (recharge), after any abstractions (withdrawals) are taken into account'. The authors also added that the base flow component of a river is assumed to equate to both aquifer discharge and recharge. This is to mention that there were no groundwater abstraction in these catchments under study and ER was calculated after subtraction of EAT and the SMD. The details about the water balance are discussed in section 5. Walmsley (2009) measured DC and DN losses from grassland and arable agricultural systems in Ireland using the amount of drainage water, being estimated from the water balance (Allen et al., 1998). Reliable estimation of the amount of water reached the GWT, generally termed as recharge, is difficult and more than one approach should normally be applied (Scanlon et al., 2002; Misstear et al., 2006). The volume of water drained from groundwater to the surface waters, W (L) was again measured using hydrological model given below (Hiscock, 2005) and compared with the amount obtained by previous measurements using the ER.

$$W = - \frac{K_{sat}(h_0^{\,2} - h_1^{\,2})}{x^2 - (\frac{L}{2})^2} \qquad (Eqn.\ 7.3)$$

where W is the volume of drainage water, h_0 is the depth of GWT bgl in the sampling well, h_1 is the water table depth of nearby river, x is the distance from groundwater divide to the well, and L is distance from the sampling well to the river where h_1 is measured.

7.4.2 Amount of dissolved C and N delivered to the surface waters

Total nitrogen includes NH_4^+, total oxidized N and DON. The weighted mean value of TN fed from groundwater to the surface waters was higher at OP and DG than at JC and SH sites in the both hydrological years (Table 7.4). The highest TN was estimated at DG (106 and 52 kg ha^{-1}, respectively in 2009 and 2010) and lowest at SH (8 and 2 kg ha^{-1}, respectively in 2009 and 2010). However, when compared between the two years, significantly lower ($p<0.05$) TN delivery was estimated in 2010 than in 2009 at all sites, possibly due to the low hydraulic loadings. Because in 2009 total rainfall was lower, this resulted in a lower ER than 2010. NO_3^- showed similar pattern between sites and between two hydrological years, as it comprised major part of TN (Table 7.4). To estimate

catchment scale N delivery from groundwater to the surface waters, there was one additional opportunity in this research to include the dissolved N_2 gas into the total DN, which was a gap so far in such N balance study. Therefore, the amount of DN delivered to the receptors including the N_2 gas was 54 and 24 kg N ha^{-1} at JC; 24 and 10 kg ha^{-1} at SH; 73 and 40 kg ha^{-1} at OP; and 109 and 54 kg ha^{-1} at DG, in 2009 and 2010, respectively. The mean DN delivered to surface waters during the two years accounted for 12, 8, 38, and 27% of the N input to the field. Walmsley (2009) measured lower leaching loses (10%) of N input via drainage in Irish grassland where the author did not include the excess N_2 which is certainly converted from NO_3^--N. Most of the DN at OP and DG sites was NO_3^--N accounting for 81% (2010) to 91% (2009) and 89% (2010) to 92% (2009), respectively. The NO_3^--N portion of DN is in good agreement with Walmsley (2009) who measured 72-92% of DN losses as NO_3^--N at arable land, and with those of Vanni et al. (2001) who estimated it in three adjacent intensively cultivated (>90% cropland) watersheds as 70-87%. Whereas, at JC and NO_3^--N fraction was less than 60% of DN and at SH it was less than 28% in both years. Very interestingly at JC and SH sites mean excess N_2 for the two years was approximately 46 and 75% of DN, suggesting that farm gate N balance without measuring the excess N_2 will potentially have a big uncertainty. However, mean excess N_2 was only 8 and 4% of DN at OP and DG sites, respectively. Unfortunately, despite the extensive research on farm gate N balance, no data on the estimation of excess N_2 were available in literature. Walmsley (2009) measured dissolved N at OP arable land at approximately 0.5 m bgl of 36 kg ha^{-1} in low tillage system to 114 kg ha^{-1} in conventional tillages system using the water balance method in 2007. This result is in agreement with the estimation of DN loses from groundwater of 40 kg ha^{-1} in 2010 to 73 kg ha^{-1} in 2009. Because, it is useful here to mention that the OP in arable land in the present research was partly under conventional tillage and partly under cover cropping system with conservation tillage. Walmsley (2009) also measured 105 kg ha^{-1} NO_3^--N loss as sampled in 0.5 m bgl in 2007 which is comparatively lower (66 kg NO_3^--N ha^{-1}) in 2009 as estimated in groundwater in the present research, suggesting that NO_3^--N can be lost via subsoils denitrification as excess N_2 before reaching groundwater. Because including the excess N_2 in groundwater, the DN was 73 kg ha^{-1}, but excess N_2 produced in subsoils below 0.5 m to the groundwater (6 m bgl) was not estimated which can be diffused upward through the soil profile.

A substantial quantity of C was lost as DOC in both years at JC and SH sites but at OP and DG sites it was significantly lower than JC and SH (Table 7.4). Groundwater was likely to

be a significant pathway of atmospheric CO_2 emissions via surface waters. It is well known that groundwater contributes to the atmospheric GHGs emissions upon discharges to the surface waters, as the dissolved gases in groundwater degasses when it reaches the surface waters. There are large uncertainties (including unaccounted DC losses) associated with the assessment of GHG budgets of croplands and a more comprehensive assessment of dominant crops and cropping systems is required, if budgets are to be truly reflective on a national and continental scale (Osborne et al., 2010). The CO_2 emissions was the lowest at OP site, which can be attributed to the low soil respiration (pers. comm. Dr. Gary Lanighan, Teagasc) and the nature of C, being observed substantial inorganic C (Chapter 3). Significantly higher CO_2 emissions at JC, SH and DG sites can be due to the input of organic C through dirty water irrigation, soiled water and other organic sources. In addition, the highest CO_2 emissions at JC can be due to the type of bedrock, being mixed with shales, which is reported to be a source of C (Schultz et al., 1980). Total DC lost as DOC, CO_2 and CH_4 showed that groundwater can be a significant pathway of terrestrial C loses, ranged from 143-344 kg ha^{-1} at JC; 78-266 kg ha^{-1} at SH; 30-89 kg ha^{-1} at OP and 116-217 kg ha^{-1} at DG during the two measurement years (2010 and 2009) (Table 10.5). The C lost as CO_2 over TC content of top soil ranged from 0.05% at OP in 2010 to as high as 0.22% at JC in 2009 (Table 10.6). Indirect N_2O emissions via groundwater ranged from 0.15 and 0.01 kg N ha^{-1} at SH to 0.34 and 0.15 kg N ha^{-1} at DG during 2009 and 2010, accounting for 0.06 and 0.01% at SH to 0.10 and 0.04% at DG of the TN input to the field (Table 7.6). The percentage of N_2O-N lost via groundwater was highest at OP showing 0.17 and 0.07% of the total N input to the field (Table 7.6).

Table 7.4Total N, NO_3^--N and DOC effluxes from groundwater to the receptors

Dissolved C and N fractions	JC		SH		OP		DG	
	2009	2010	2009	2010	2009	2010	2009	2010
ER (mm y^{-1})	836	385	759	326	587	296	674	326
TN (kg N ha^{-1})*	34	15	8	2	69	36	106	52
NO_3^- (kg N ha^{-1})	33	13	7	2	66	32	100	47
DOC (kg ha^{-1})	28	12	12	5	6	2	7	3
DON (kg ha^{-1})	1.4	1.4	1.7	0.3	2.9	4.3	5.3	5.4
Denitrified N_2-N	18	9	15	8	4	3	3	2
DN (kg N ha^{-1})*	52	24	24	10	73	40	109	54

*TN includes total NH_4^++TON+DON; DN includes NH_4^++TON+DON+ denitrified N_2

Table 7.5 Annual GHGs emissions from groundwater to the receptors at four sites

Dissolved gases	JC		SH		OP		DG	
	2009	2010	2009	2010	2009	2010	2009	2010
N_2O-N (kg ha^{-1})	0.28	0.06	0.15	0.01	0.25	0.10	0.34	0.15
Mean for 2 years (kg ha^{-1})	0.17		0.08		0.18		0.24	
CO_2-C (kg ha^{-1})	314	130	254	73	83	28	210	113
Mean for 2 years (kg ha^{-1})	222		164		56		162	
CH_4-C (kg ha^{-1})	1.73	1.07	0.09	0.14	0.04	0.01	0.01	0.01
Mean for 2 years (kg ha^{-1})	1.40		0.12		0.03		0.01	

Table 7.6 Dissolved N_2O; and CO_2 and CH_4 losses (%) of respectively N in to the field, and TN and TC content in top soil

Dissolved gases	JC		SH		OP		DG	
	2009	2010	2009	2010	2009	2010	2009	2010
N_2O-N lost (%)†	0.09	0.02	0.06	0.01	0.17	0.07	0.10	0.04
N_2O-N lost (%)‡	0.56	0.28	0.69	0.13	0.36	0.22	0.33	0.27
N_2O-N lost (%)**	0.003	0.001	0.001	0.001	0.003	0.002	0.003	0.001
CO_2-C lost (%)*	0.22	0.13	0.10	0.06	0.07	0.05	0.14	0.08
CH_4-C lost (%)*	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

†calculated from annual N_2O-N delivered to receptors divided by TN input to grassland
‡ calculated from annual N_2O-N delivered to receptors divided by DN delivered to receptors
**calculated from annual N_2O-N delivered to receptors divided by TN content in top soil
*calculated from weighted mean of dissolved CO_2-C and CH_4-C divided by TC in top soil

However, when compared with the calculated amount of drainage water, the ER in 2009 was approximately similar to the volume of calculated drainage water at JC and SH, whereas the ER in 2010 was significantly lower than the drainage volume at all sites (Table 7.7). But the volume of drainage water in 2009 was almost similar to 2010 (Table 7.7), suggesting that groundwater zone is under certain pressure which is balanced with the pressure of ER that moves downwardly and another from deeper aquifer water that moves upwardly. In 2010 (a comparatively dry year), the higher amount of calculated drainage water than the ER suggesting that groundwater from deeper layer below the sampling depth contributed to the drainage water due to the confined nature of the aquifer, such as bedrock zone in particular. On the contrary, higher drainage volume in 2009 than 2010 at OP and DG sites indicating two possibilities: one is that a direct discharge of rainwater to the river can be possible especially during the heavy rainfall event, and another is that due to their free draining nature the actual rise in water table during heavy rainfall event is missed, being sampled on monthly basis. It can happen due to the comparatively thicker unsaturated zone with higher permeability than other two sites (Chapter 5). At JC and SH sites almost equal ER and calculated amount of drainage water implies that direct discharge in these two sites is zero because it is argued that in the low permeable subsoils, direct discharge to the streams is insignificant (Misstear et al., 2008). Therefore, to

estimate nutrient discharge, estimation of amount of water delivered to river based on ER and drainage water calculation model should carefully consider all these pathways of water that are being delivered to the streams. Misstear and Fitzsimons (2007) highlighted the need for a good conceptual understanding of the various pathways that contribute flow to the river. However, the approximately similar ER to the calculated drainage water in 2009 showed that estimation of nitrate delivery from groundwater to river should be a close approximation of NO_3^- delivery to the streams in the study areas. Based on the drainage calculation, the amount of DN losses from JC, SH, OP and DG in 2009 were 17, 10, 27 and 25% of the total N input, respectively (Table 7.7). In 2010, the DN losses were approximately similar to 2009 accounted for 16, 10, 27 and 23% of the N input. The NO_3^--N accounted for 11, 3, 24 and 24% of the total N input, respectively at JC, SH, OP and DG, which was 9, 2, 22 and 19% in 2010.

Table 7.7 The C and N losses from groundwater to the surface waters based on the calculated volume of drainage water for the year 2009 and 2010

Items	JC		SH		OP		DG	
	2009	2010	2009	2010	2009	2010	2009	2010
Drain. (mm y^{-1})*	837	827	755	725	322	310	557	481
TN (kg N ha^{-1})*	34	31	8	5	38	38	87	77
NO_3^--N (kg ha^{-1} y^{-1})	33	28	7	4	36	34	83	69
DOC (kg ha^{-1} y^{-1})	24.3	24.0	10.6	8.7	2.9	2.5	5.6	3.8
DON (kg ha^{-1})	1.4	2.9	1.3	0.6	1.7	4.5	4.4	7.9
Denitrified N_2-N	18	20	15	18	2	3	2	3
DN (kg N ha^{-1})	52	51	23	23	40	42	90	80
CO_2 (kg C ha^{-1} y^{-1})	315	280	252	162	45	29	174	167
CH_4 (kg C ha^{-1} y^{-1})	1.73	2.31	0.09	0.31	0.02	0.1	0.01	0.00

*mean ± SE; **dissolved nitrogen (N_2O+N_2+NO_3^--N+DON, dissolved organic N)

7.4.3 Evidence of in situ GHGs productions in groundwater revealed by push-pull method

There was an interesting question to scientists (Groffman et al., 1998) that whether these gases are being produced in groundwater or are they leached out only from the top soil. An in situ push pull method was applied to investigate the sources of N_2O and excess N_2 in groundwater (Chapter 6). It was observed that significant amount of ^{15}N-enriched NO_3^--N was converted in situ in groundwater to N_2O and N_2. Concurrent productions of CO_2 and CH_4 concentrations together with N_2O were showed in subsoil, interface and bedrock at JC and at interface and bedrock at OP (no water in subsoil in OP during the experiment) (Figure 7.19). The CO_2 production was observed in both sites in all depths but there was no

CH_4 production at OP site, may be due to the aerobic conditions of the site (DO concentration > 6.5 mg L^{-1}). Xu et al. (2009) summarised that NO_3^- injection in soil solutions in 60 cm bgl under a temperate old-growth forest increased dissolved N_2O production with simultaneous production of dissolved CO_2. These results suggest that N_2O, CO_2 and CH_4 can be produced in situ in groundwater under favourable environmental conditions where they can also be transformed simultaneously to other forms such as N_2O-N_2 and CO_2-CH_4, in particular. Another implication of such measurement is that during denitrification consumption of C to produce CO_2 take place in groundwater. These findings were in line with Xu et al. (2009) who noted that denitrification after NO_3^- addition decreased DOC concentrations. However, such CO_2 emissions via groundwater beneath agricultural systems is not considered as important because CO_2 production and consumption in agricultural ecosystems are generally balanced (Minamikawa et al., 2010).

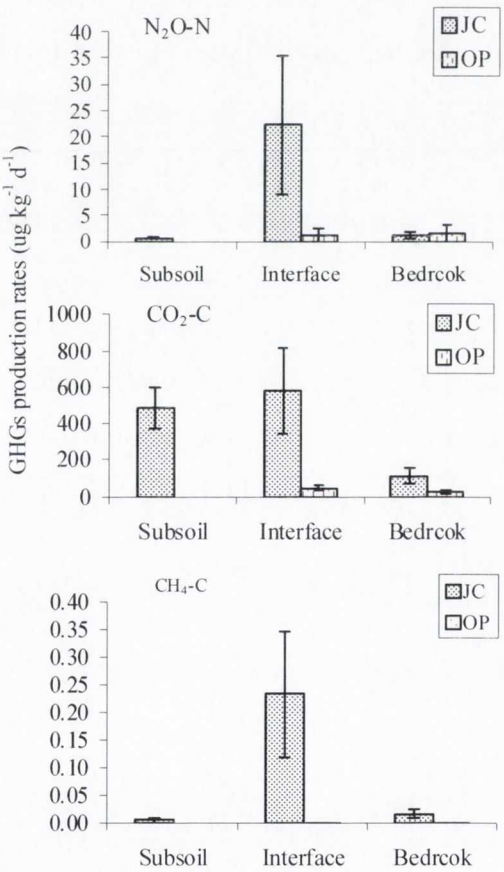


Figure 7.19In situ productions of N_2O, CO_2 and CH_4 in groundwater in subsoil, interface and bedrock at JC and at interface and bedrock at OP

7.4.4 Conclusions

Dissolved C and N delivery to the surface waters via groundwater cause a substantial amount of C and N loses from the terrestrial ecosystems. However, the extent of loses depends on the land use (rates of N inputs) and the hydrogeological environments. For instances, in grassland dairy farming systems the DN loading from terrestrial ecosystems to the aquatic ecosystems ranged from 10% in poorly drained clay soils to 30% in free draining sandy loam soils. In the arable land cultivated partly with conventional and conservation tillage systems with well drained sandy loam soils, the DN loadings to the surface waters was approximately 38% of N input. The amount of DC loadings to surface waters via groundwater was not significant in compare to the TC content of top soil. However, as a major GHG, CO_2 emissions via groundwater ranged from 29-315 kg C $ha^{-1}y^{-1}$, irrespective of sites can be expected to be an important source of atmospheric CO_2 build up. The CH_4 emissions via groundwater from the terrestrial ecosystems do not seem to be an important issue because it appears to be produced in groundwater in small amount but very intermittent over time and space. Indirect N_2O emissions via groundwater denitrification account for approximately 0.10 to 0.20% of N input, which seem to be an important component of atmospheric N_2O emissions. Because, indirect emissions (0.10-0.20% of N input) can be approximately 10-20% of the surface N_2O emissions. Nonetheless, N_2O has global warming potential 300 times of CO_2. Therefore, estimation of indirect N_2O emissions via groundwater denitrification is crucial. Another biogeochemical implication of measurement of GHGs in groundwater is that C and N are being processed in groundwater at varying depths and GHGs are being produced in situ in groundwater.

CHAPTER 8. SUMMARY AND CONCLUSIONS

8.1 Hydrologic regimes of the study sites

The study period (Feb, 2009-Jan, 2011) had two contrasting hydrological years with 130-140% in 2009 and 85-90% in 2010 of the average rainfall and ER. Interestingly, during the SMD period dissolved N_2O in groundwater was low but excess N_2 was higher than other sampling times of the year, suggesting that denitrification in groundwater, is an in situ process. An in situ push-pull test supported the hypothesis that denitrification is an in situ process in groundwater. Low permeable sites coupled with shallow unsaturated zone had lower NO_3^- concentrations in groundwater than the high permeable sites, suggesting the potential of low permeable sites to natural attenuation of NO_3^-.

8.2 Denitrification Potential in Subsoils at Grassland

Subsoil denitrification substantially reduces landscape NO_3^- delivery to groundwater and indirect N_2O emissions to the atmosphere. N_2O emissions were higher from the A than the B and C horizons, and were significantly lower from soils that received only NO_3^- than soils that received NO_3^- + either C source. During 17-day incubation, TND losses of the added N decreased significantly with soil depth and were increased by the addition of either C source. The ratios of N_2O to N_2O+N_2 were higher in the A than in the deeper horizons in B and C, indicating the potential of subsoils for a more complete reduction of N_2O to N_2. Stepwise multiple regression analysis revealed that N_2O flux increased with TOC and TN, but decreased with NO_3^--N which together explained 88% of the variances. The results suggest that without C addition, potential denitrification rate below the rooting zone is low. Subsoil denitrification can be accelerated either through introducing C directly into permeable reactive barriers and/or indirectly, by irrigating soiled water and manipulating agricultural plant composition and diversity.

8.3 Groundwater Geochemical Properties and NO_3^- Distributions

This study focuses on the spatio-temporal variability in groundwater hydrogeochemistry in light with its potential implication on the distributions of NO_3^--N across shallow to deep

groundwaters in diverse landscape settings. The NO_3^- concentrations varied significantly between sites and significantly decreased with increasing depths. A decreased NO_3^- concentration was consistent with an increased DOC, Mn^{2+}, Fe^{2+}, NH_4^+, and CH_4, but with decreased K_{sat}, depth of unsaturated zone, DO, Eh and S^{2-} concentrations, suggesting the occurrence of biological NO_3^- reduction in groundwater. Groundwater can be a major pathway of terrestrial C loss as DOC, dissolved CO_2 (12-35 mg C L^{-1}) and CH_4 (1-246 µg C L^{-1}). Multiple linear regressions following stepwise method revealed negative relationship between NO_3^- and logDOC and logCH_4 but positive relationship with logEh and logK_{sat}, explaining 74% of the variances of log-transformed NO_3^- concentration.

8.4 Natural NO_3^- Attenuation via Denitrification across Sites

This study focused on the quantifications of when, where and how much NO_3^- retained by denitrification with context to its end products (N_2O and N_2). Denitrification was found to be a significant pathway of groundwater NO_3^- reduction at low permeability sites causing 46-77% losses of initial NO_3^--N, leached to groundwater, resulting in a net NO_3^--N of 1-4 mg N L^{-1}. Conversely, high permeability sites caused 4-8% losses resulting in a net NO_3^--N of 11-15 mg N L^{-1}. Mean N_2O emission factors (EF5-g) across sites were considerably higher (0.0032-0.0044) than the IPCC default (EF5-g 0.0025). Multiple electron donors (both organic C and Fe/S minerals) coupled with low Eh (<100 mV), DO (<2 mg L^{-1}) and permeability (K_{sat}<0.005 m d^{-1}); and shallow unsaturated zone (<2 m bgl) created denitrification 'hot spots'.

8.5 In Situ Denitrification Capacity and DNRA Rates in Grassland vs. Arable Land Measured by Push-pull Method

The N2O and N2 production via denitrification occurs in situ in groundwater and differed significantly between sites. In situ denitrification rates were higher at bedrock-interface than in subsoil and bedrock, where the later two were similar, indicating that denitrification processes is not limited to shallow groundwater; rather, it is an important process of NO_3^- conversion to N_2O and N_2 in shallow to deep groundwaters along groundwater flow paths. Denitrification rates were positively correlated with ambient DOC but negatively and significantly correlated with the ambient DO, Eh, K_{sat} and NO_3^-. In situ push-pull method

can give insights into the fate and transformations of NO_3^- in shallow to deep groundwaters including its major end products either reactive forms and benign form (N_2).

8.6 In situ denitrification in shallow groundwater beneath spring barley - cover crop rotation

Introduction of cover crop e.g., mustard into the agricultural management activities appeared to enhance denitrification process mainly by adding more organic C than the plot which has not been cropped with any cover crop. In addition, the end product of denitrification process was mainly N_2 (~100%), suggesting that inclusion of cover crop with spring barley can reduce indirect N_2O emissions to the atmosphere. These results suggest that enhanced C concentrations into the sediments and groundwater beneath arable systems can reduce NO_3^- contamination in ground and surface waters and indirect N_2O emissions to the atmosphere.

8.7 Dissolved C and N losses from Terrestrial to Aquatic Ecosystems

Substantial amount of DC and DN are delivered from terrestrial ecosystems to the surface waters via groundwater. However, the extent of losses depends on the land use, N input and the hydrogeological environments. A large portion of the DN is N_2 in low permeability sites, but NO_3^- in high permeability sites. As a major GHG, CO_2 emissions via groundwater can be an important source of atmospheric CO_2 build up. The CH_4 emissions via groundwater from the terrestrial ecosystems are low with high temporal variability. Indirect N_2O emissions via groundwater account for approximately 10-20% of the total N_2O emissions. Nonetheless, N_2O has global warming potential 300 times of CO_2. Therefore, estimation of indirect N_2O emissions via groundwater denitrification is crucial.

8.8 Optimization of Dissolved CO_2, CH_4 and N_2O Extraction Method

He: water ratio, shaking times and standing times showed significant influences on GHGs concentrations. Response surface methodology was used to determine if there was an

optimum operating point contained within the ranges of these factors. The optimum shaking time was approximately 13 minutes. Extrapolations were required for ratio and standing time, being 4.4, 3.4 and 3.0; and 63, 108 and 17 min for N_2O, CH_4 and CO_2, respectively.

8.9 Conclusions

From the environmental and health concern for excess reactive N across the globe, it is now crucial to understand the biogeochemical attenuation of NO_3^- rather than only hydrogeological characterizations to estimate the vulnerability of aquifer. Because achieving the target of water framework directive, NO_3^- directive and Kyoto protocol, mitigation of excessive reactive N delivery to the environment and N_2O emissions to the atmosphere is of utmost importance to the scientists and the policy makers which requires accurate and extensive estimation of all the species of N and understanding of their biogeochemical transformations in the environment.

Subsoil denitrification rates were low at ambient C levels. Addition of C significantly increased denitrification rates. The main product of denitrification was N_2 in subsoils. Denitrification potentials were mainly regulated by substrates including total organic C, total N and TON. The findings suggest that both glucose-C and DOC were highly effective for the complete reduction of NO_3^- to occur in subsoil environments and subsoils could have a large potential to attenuate NO_3^- that has leached below the rooting zone, with the production of more N_2 than N_2O, if available C is not limiting.

Denitrification is the single most important pathway of NO_3^- removal in groundwater and becomes a permanent sink when its end product is N_2. In the terrestrial ecossytems, mainly in groundwater beneath the agricultural systems, the estimation of N_2 production was ignored due to the methodological difficulties (arised from its high background concentrations). The different species of N including dissolved N_2O and N_2 were measured in groundwater over two years from Feb, 2009 to Jan, 2011 to understand the fate and transformation of N in the existing environmental conditions. The concurrent dynamics in biogenic C was also considered by measuring the DOC, CO_2 and CH_4 concentrations in groundwater. The details hydrogeochemical characterisations of groundwater were conducted to understand the implications of ambient hydrogeochemical properties on the N

biogeochemistry. Abundances of denitrifier functional genes were analysed to make a complete scenario of denitrification process in groundwater. Long-term monitoring of denitrification was found to be a significant pathway of NO_3^- removal in some sites (36-68%, of the NO_3^- delivered to groundwater, respectively at JC and SH) but not at other sites (3-6%, respectively at OP and DG sites). From an in situ ^{15}N tracer test it was observed that DNRA contributed to 2% at JC to 38% at OP of the total biogeochemical NO_3^- losses (denitrification plus DNRA). The total losses via denitrification plus DNRA were 25 and 0.5% of the injected NO_3^- per day at JC and OP site, respectively, which suggesting the high potential of JC site to remove NO_3^- in groundwater. The DNRA therefore, indicating the production of NH_4^+ in groundwater which can further undergoes fixation and/or microbial assimilation because plant uptake in such depth of groundwater is unrealistic. However, the long-term in situ estimation of NH_4^+ showed the evidence of accumulation of NH_4^+ in groundwater and its delivery to surface waters. In general there were higher denitrification and DNRA rates in bedrock interface than subsoil and bedrock. Unlike the long-term monitoring, the higher N_2O/N_2O+N_2 during short incubation suggest that N_2O undergoes further reduction while passing through and from groundwater to surface waters. The contrasting denitrification rates between sites suggest that denitrification can be an important sink for NO_3^- and N_2O in some sites, but not in others, depending on hydrogeologic conditions. Bedrcok-interface in groundwater is likely to be a hot spot to reduce the NO_3^- passing at some sites. The strong variation with hydrogeologic conditions suggests predictability and mapping of sites with significant subsurface denitrification capacity.

Annual dissolved N fluxes were 8-12% of N input in poorly drained sites which were 27-38% in free drained sites. However, in free drained site main N species was NO_3^--N (80-90% of DN) which was 26-60% in poorly drained site. Indirect N_2O emissions was likely to be an important component of the atmospheric N_2O emissions in these study sites, being 0.10 at JC and SH sites to 0.20% at OP and DG sites of the applied N, which is equivalent to approximately 10-20% of the total atmospheric N_2O emissions. N_2O emission factors across sites showed a higher emission factor than the IPCC default value which was even much higher in the poorly drained sites coupled with shallow GWT than the free drained sites.

Denitrification functional genes were present in all sites and depths in similar concentrations, suggesting that hydrogeological properties are the main controlling factors

of groundwater denitrification. The DO and Eh seem to be the main controlling factors in groundwater where multiple electron donors like DOC and Fe/Mn and sulphur compounds are available. Low permeable area with shallow groundwater table showed significant amount of NO_3^- removal in groundwater and vice versa. Dirty water irrigation in a low permeable site created denitrification 'hot spot' in groundwater and removed 100% of NO_3^- delivered to groundwater.

8.10 Recommendation for future research

These studies showed clear differences in denitrification capacity between landscape settings and could easily form the basis for national or continental scale assessments of landscape vulnerability to NO_3^- pollution. The fate of groundwater N_2O requires further research to quantify the actual emissions to the atmosphere during groundwater transport. Any upward emissions of N_2O and N_2 from the groundwater to the unsaturated zone and to the atmosphere are of particular importance for catchment scale N_2O and N balance and to have precise estimates of denitrification. Predicting the spatial distribution of denitrification zones in Irish aquifers will help refine indirect N_2O emissions and NO_3^- reduction potential for air and water quality risk assessment. These studies could also form the base for improved methodologies for quantifying indirect N_2O emissions and evaluating mitigation strategies. In situ denitrification rate by ^{15}N-enriched NO_3^- incubation in different seasons is necessary to have better understanding of the rates of NO_3^- reduction. Landscape engineering with denitrification trench/wall or simulating riparian wetlands can be of interest to promote denitrification. Permeable reactive barrier (PRB) to supply electron sources like C, metal bound sulphur (Fe/Mn) can be useful to reduce the risk of agricultural NO_3^- delivery to surface waters and N_2O emissions to the atmosphere. It would be an interesting idea to microbial, and/or hydrogeochemical parameter (DO, Eh, residence time, DOC, Fe/Mn/S minerals) based mapping up of national NO_3^- vulnerability zone.

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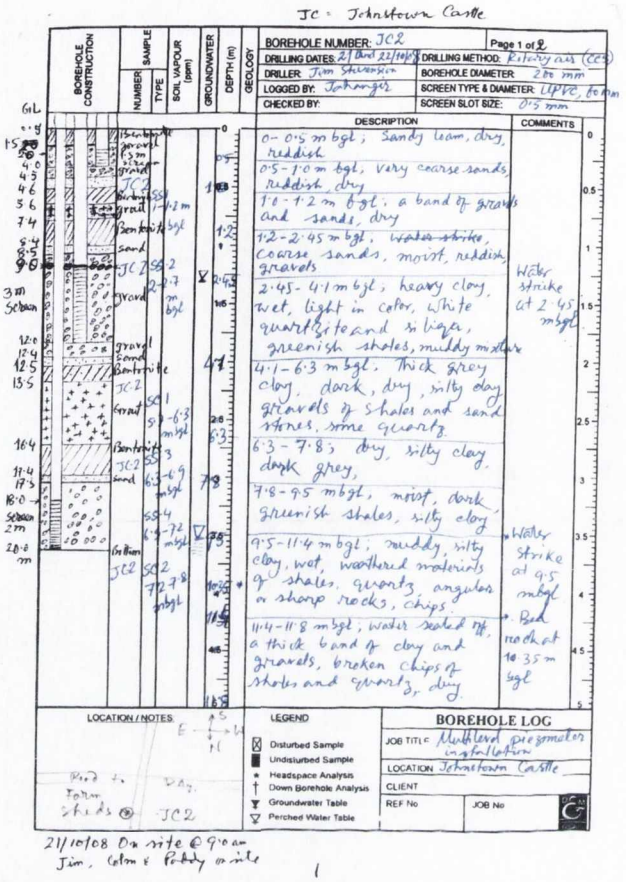
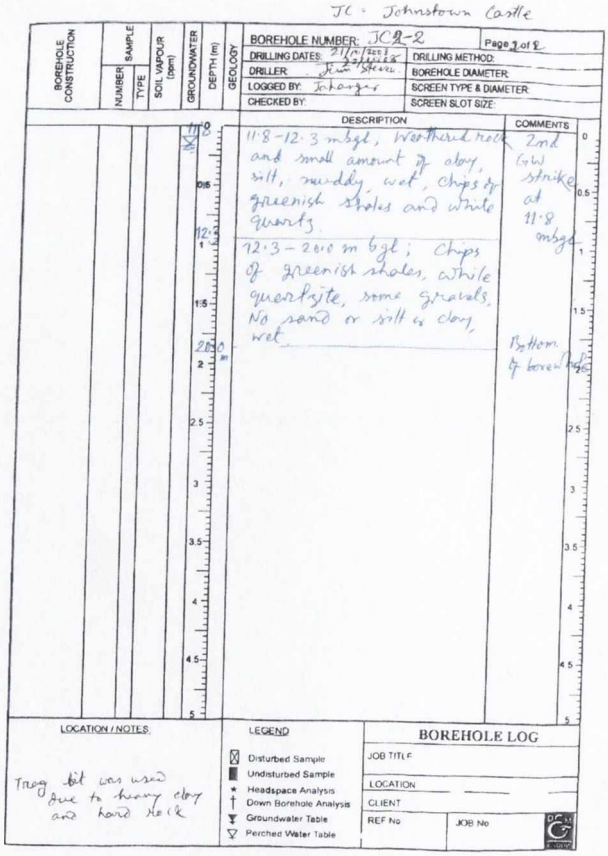
APPENDICES

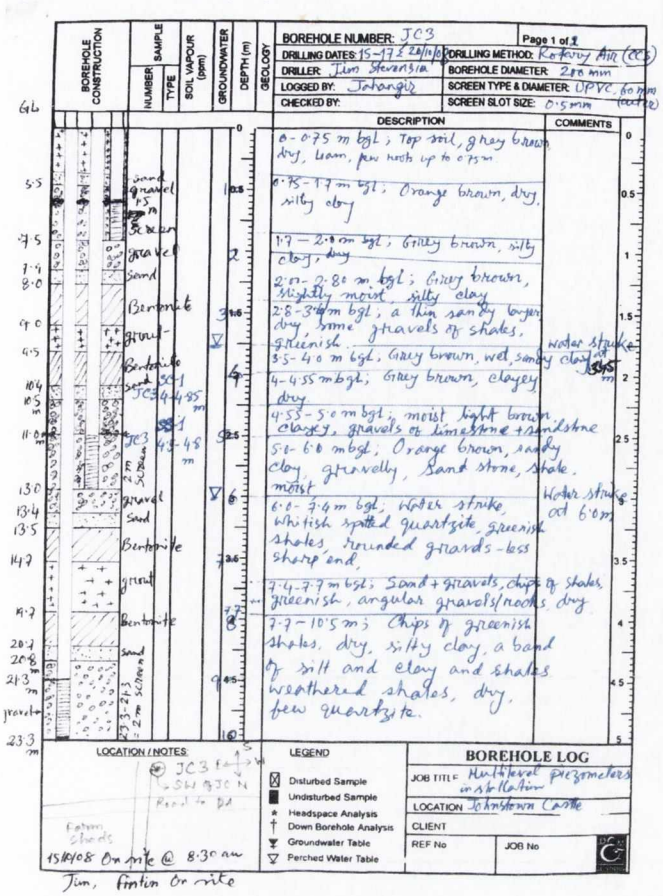
Appendix 1



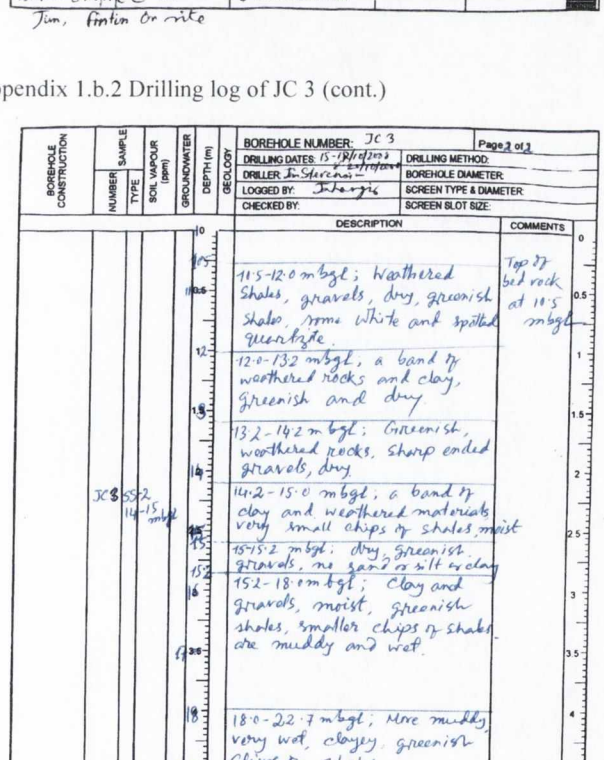
Appendix 1.a.2 Drilling log of JC 2 (cont.)



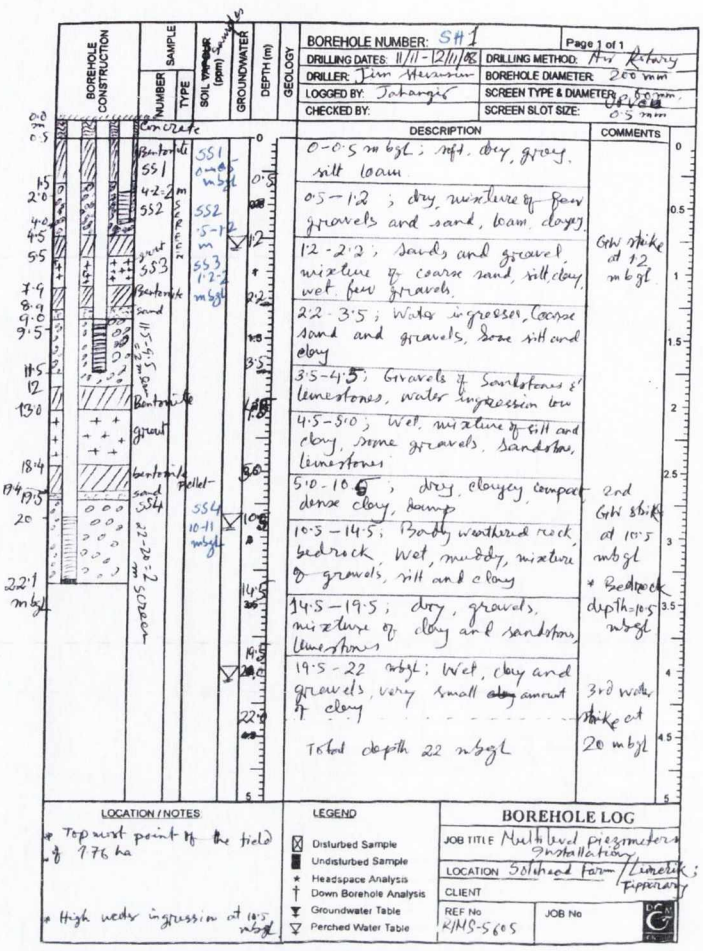
Appendix 1.b.1 Drilling log of JC 3



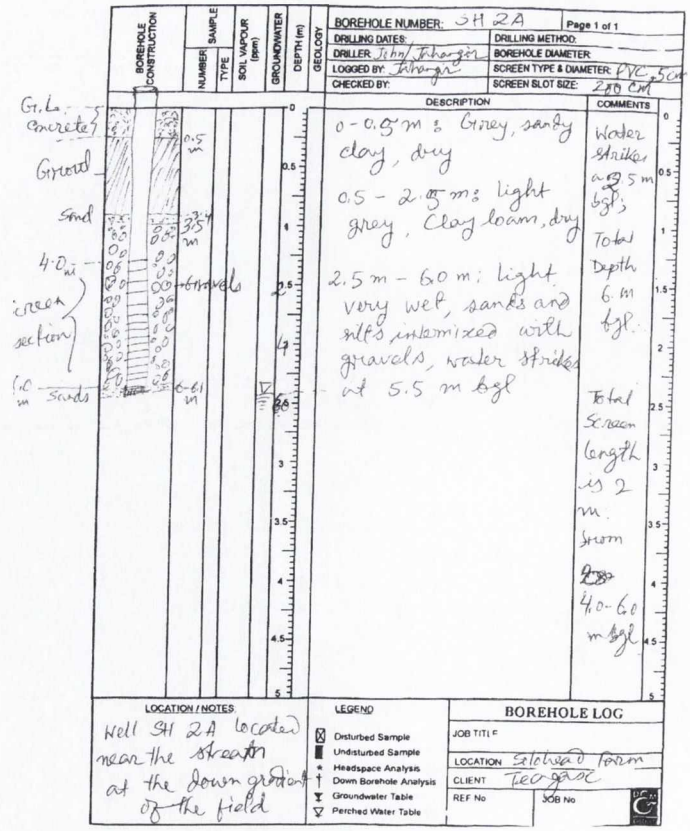
Appendix 1.b.2 Drilling log of JC 3 (cont.)



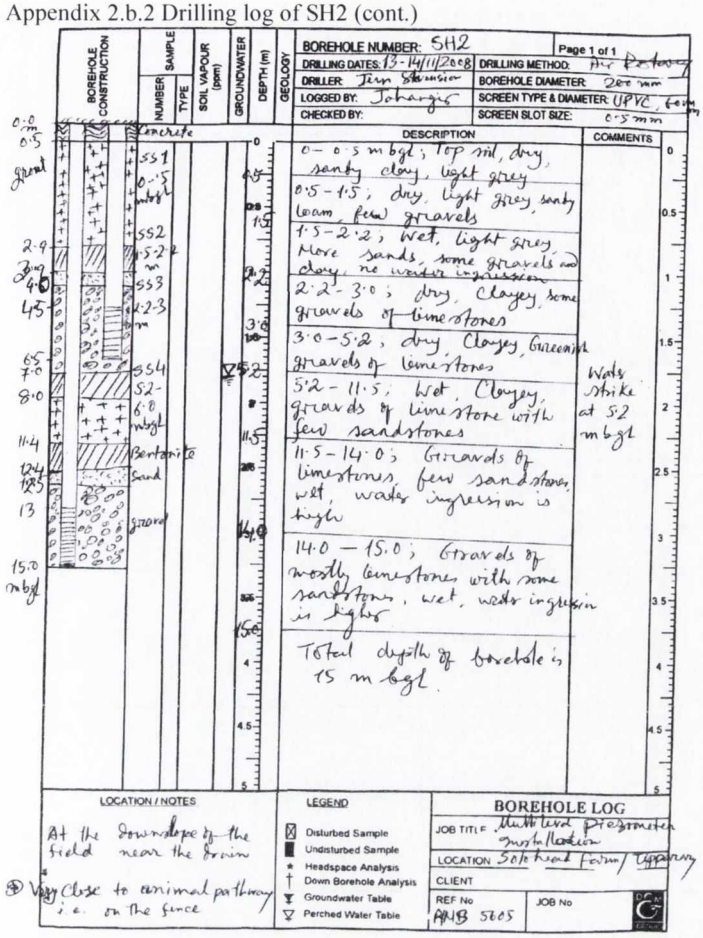
Appendix 2.a Drilling log of SH1



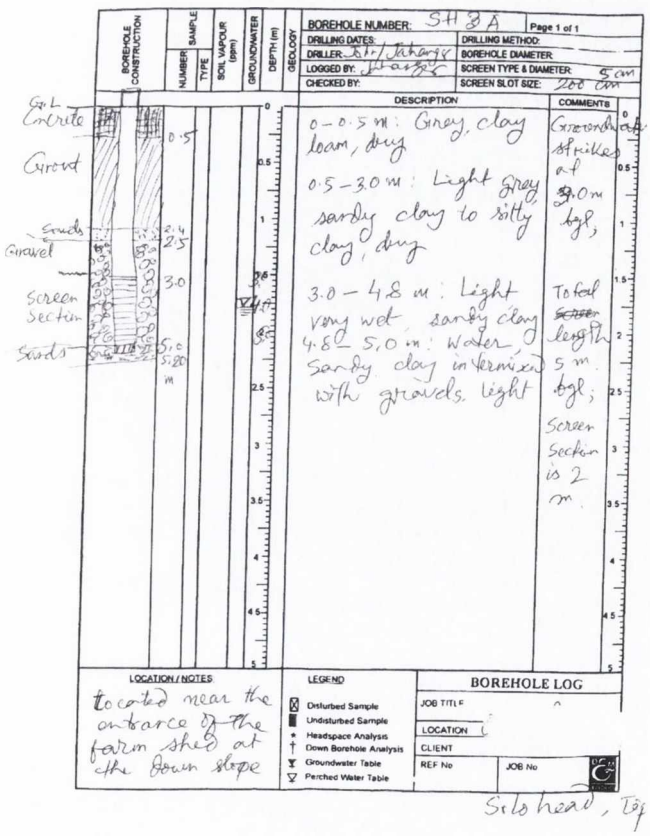
Appendix 2.b.1 Drilling log of SH2



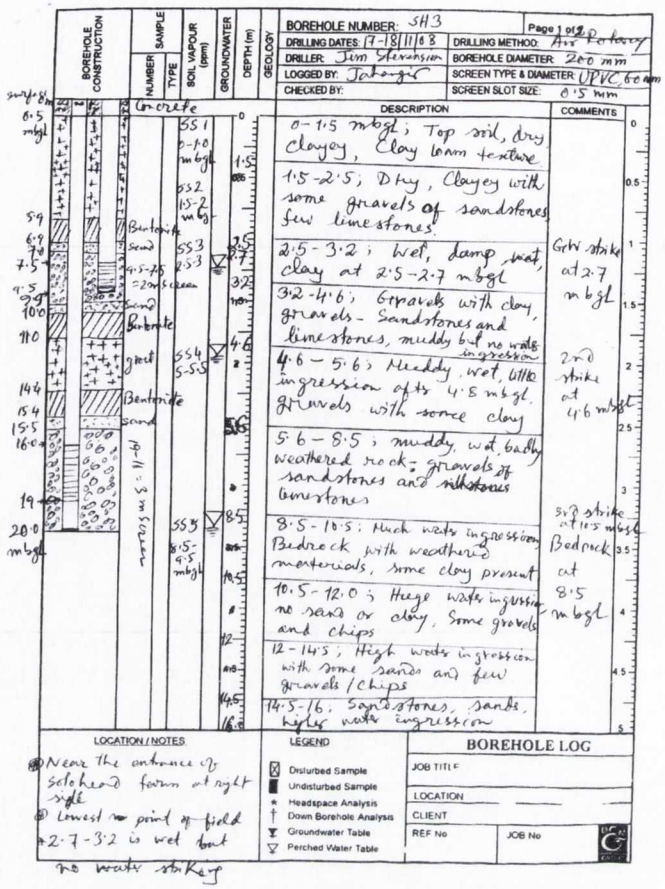
Appendix 2.b.2 Drilling log of SH2 (cont.)



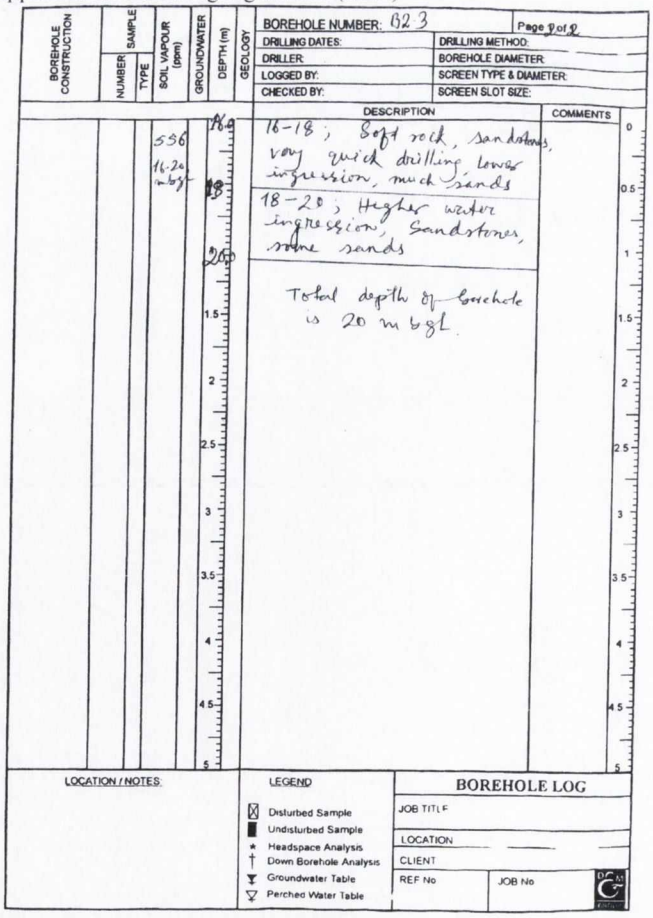
Appendix 2.c.1 Drilling log of SH3



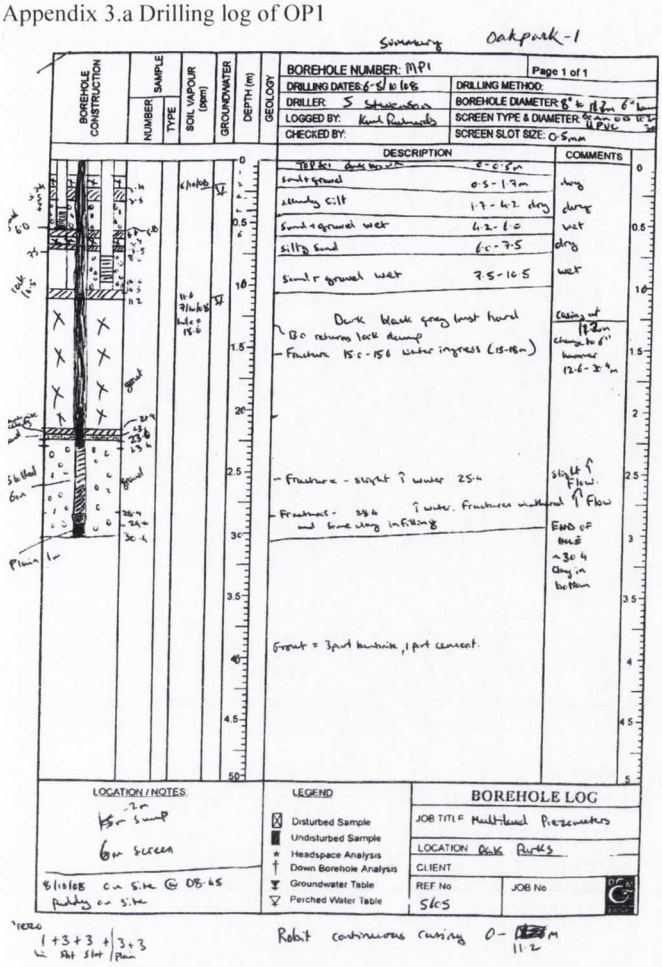
Appendix 2.c.2 Drilling log of SH3 (cont.)



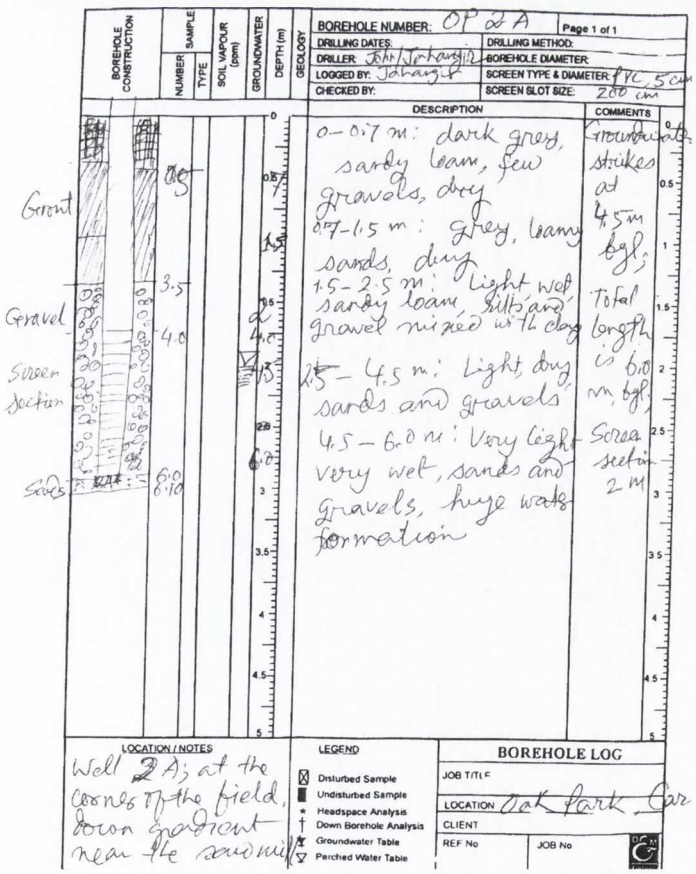
Appendix 2.c.3 Drilling log of SH3 (cont.)



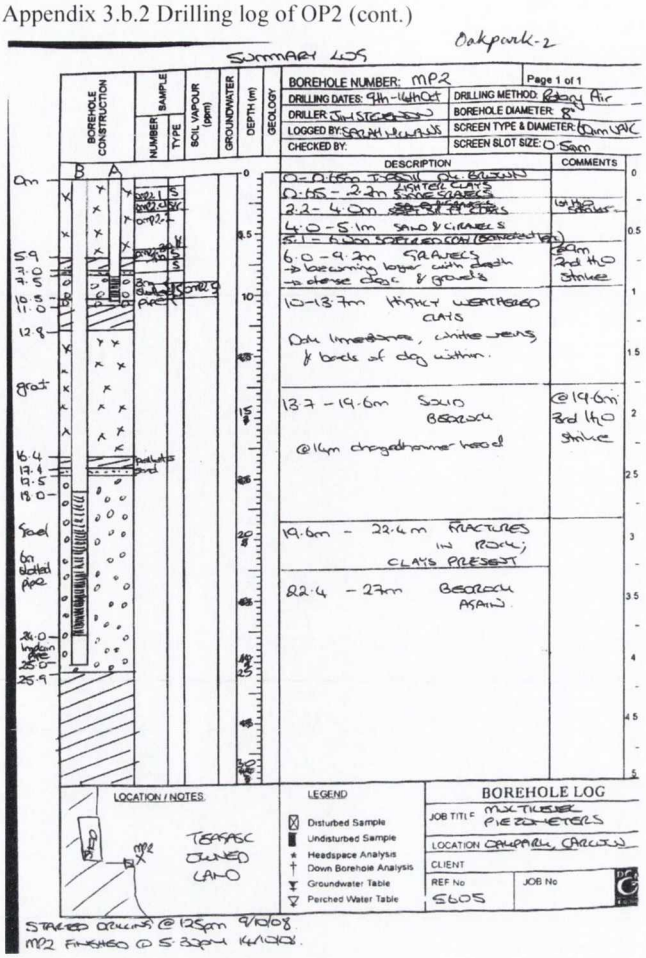
Appendix 3.a Drilling log of OP1



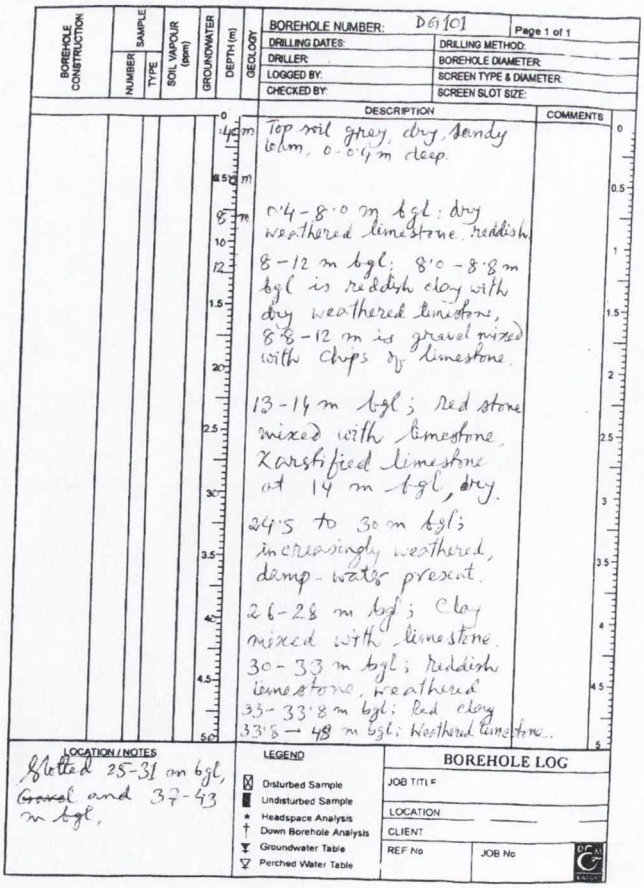
Appendix 3.b.1 Drilling log of OP2



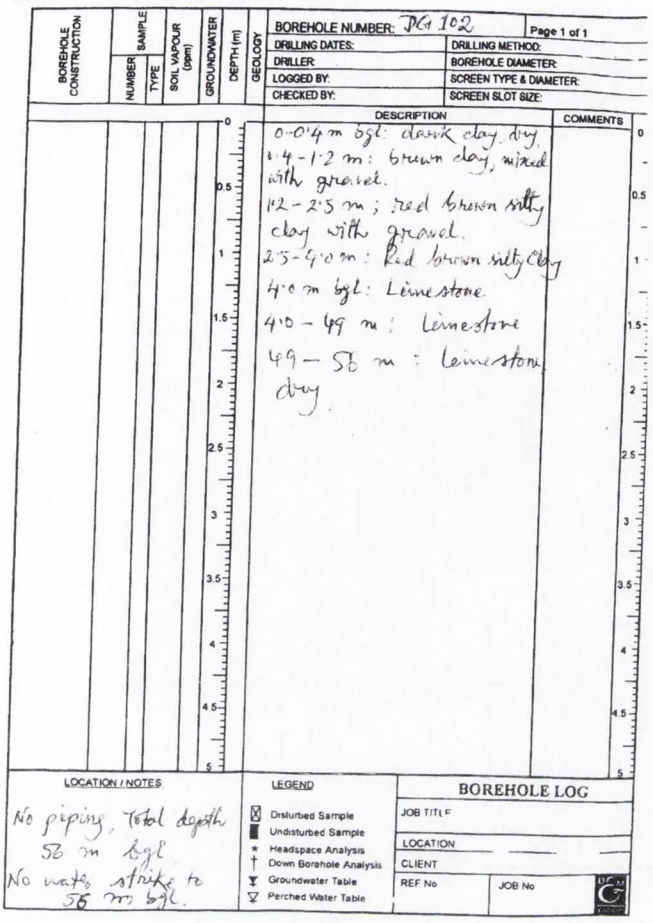
Appendix 3.b.2 Drilling log of OP2 (cont.)



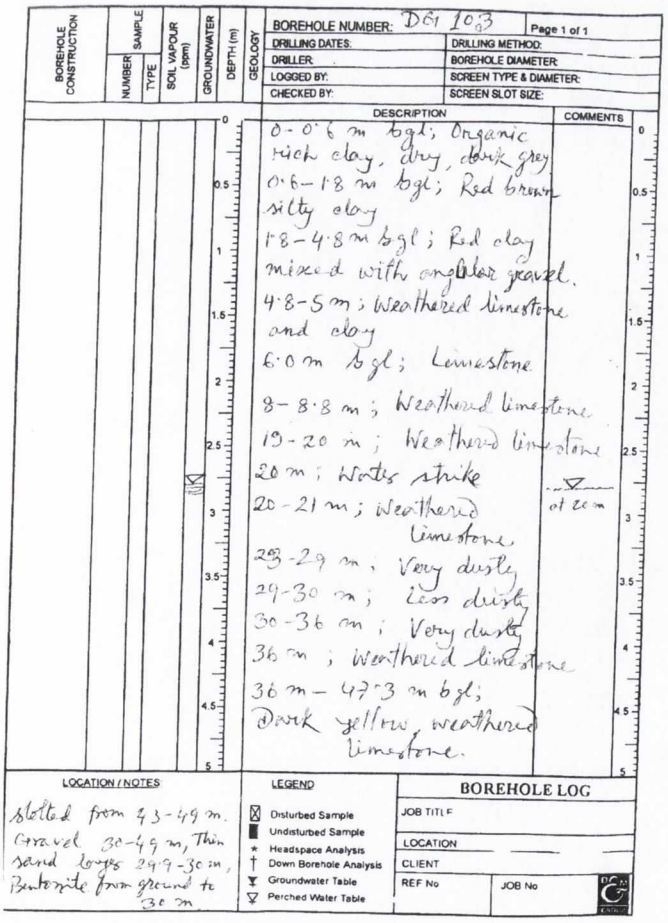
Appendix 4.a Drilling log of DG101



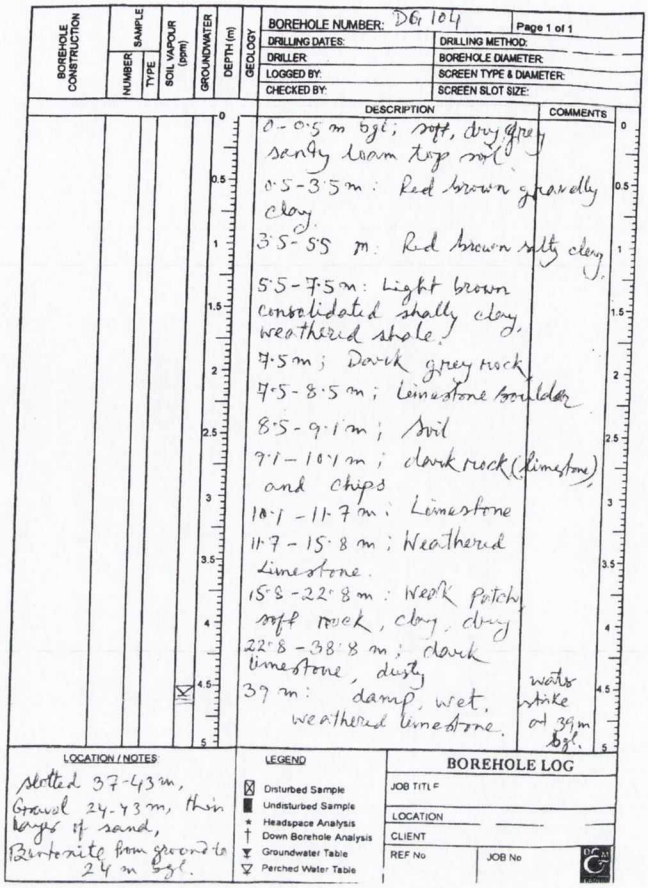
Appendix 4.b Drilling log of DG102



Appendix 4.c Drilling log of DG103



Appendix 4.d Drilling log of DG104



BOREHOLE NUMBER: DG 104 Page 1 of 1

DRILLING DATES: | DRILLING METHOD:
DRILLER: | BOREHOLE DIAMETER:
LOGGED BY: | SCREEN TYPE & DIAMETER:
CHECKED BY: | SCREEN SLOT SIZE:

BOREHOLE CONSTRUCTION | SAMPLE NUMBER | SAMPLE TYPE | SOIL VAPOUR (ppm) | GROUNDWATER | DEPTH (m) | GEOLOGY | DESCRIPTION | COMMENTS

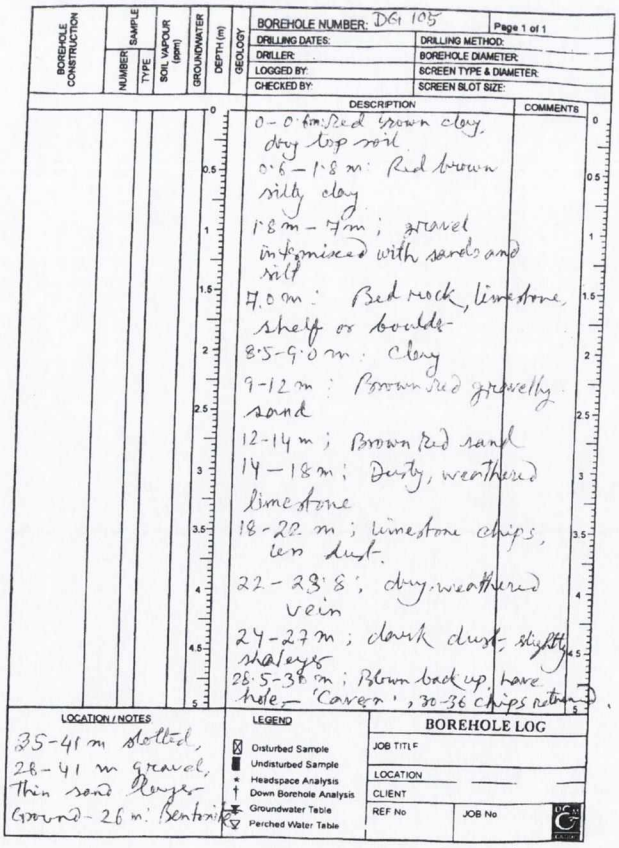
0 - 0·5 m bgl; soft, dry grey sandy loam top soil
0·5 - 3·5 m: Red brown gravelly clay.
3·5 - 5·5 m: Red brown silty clay.
5·5 - 7·5 m: Light brown consolidated shally clay, weathered shale.
7·5 m; Dark grey rock.
7·5 - 8·5 m; Limestone boulder
8·5 - 9·1 m; Soil
9·1 - 10·1 m; dark rock (limestone) and chips
10·1 - 11·7 m: Limestone
11·7 - 15·8 m; Weathered Limestone.
15·8 - 22·8 m: Weak patch, soft rock, clay, dry
22·8 - 38·8 m; dark limestone, dusty
39 m: damp, wet, weathered limestone.
water strike at 39 m bgl.

LOCATION / NOTES:
slotted 37-43 m,
Gravel 24-43 m, thin layer of sand,
Bentonite from ground to 24 m bgl.

LEGEND:
Disturbed Sample
Undisturbed Sample
Headspace Analysis
Down Borehole Analysis
Groundwater Table
Perched Water Table

BOREHOLE LOG
JOB TITLE
LOCATION
CLIENT
REF No | JOB No

Appendix 4.e Drilling log of DG105



BOREHOLE NUMBER: DG 105 Page 1 of 1

DRILLING DATES: | DRILLING METHOD:
DRILLER: | BOREHOLE DIAMETER:
LOGGED BY: | SCREEN TYPE & DIAMETER:
CHECKED BY: | SCREEN SLOT SIZE:

BOREHOLE CONSTRUCTION | SAMPLE NUMBER | SAMPLE TYPE | SOIL VAPOUR (ppm) | GROUNDWATER | DEPTH (m) | GEOLOGY | DESCRIPTION | COMMENTS

0 - 0·6 m: Red brown clay, dry top soil
0·6 - 1·8 m: Red brown silty clay
1·8 m - 7 m; gravel interspersed with sands and silt
7·0 m: Bed rock, limestone shelf or boulder
8·5 - 9·0 m: Clay
9 - 12 m: Brown red gravelly sand
12 - 14 m; Brown red sand
14 - 18 m; Dusty, weathered limestone
18 - 22 m; limestone chips, less dust.
22 - 23·8; dry, weathered vein
24 - 27 m; dark dust, slight shatter
28·5 - 36 m; Blown back up, have hole - 'Cavern', 30-36 chips returned.

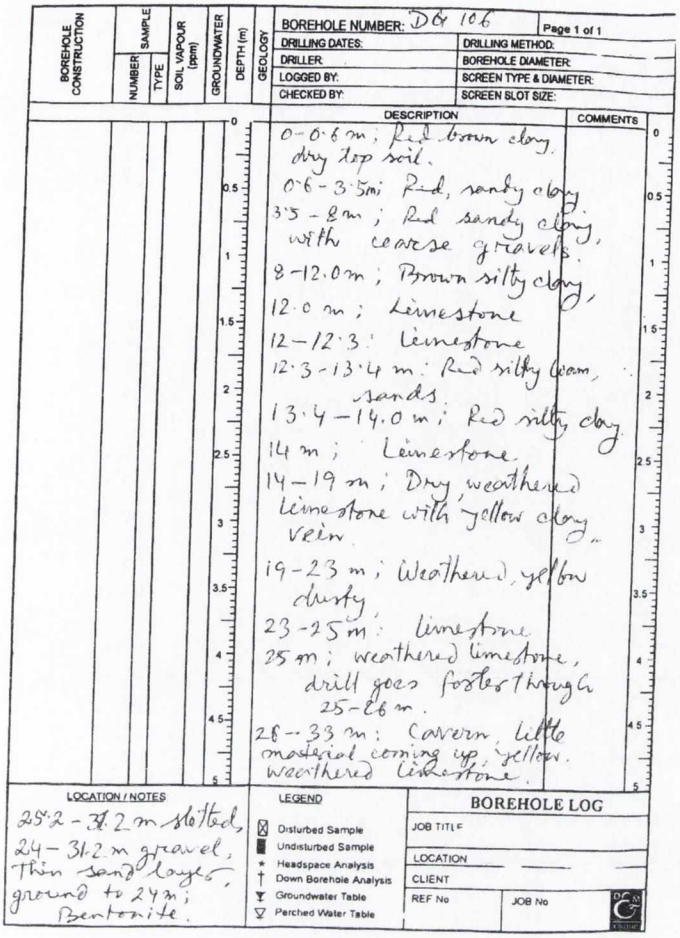
LOCATION / NOTES:
35-41 m slotted,
28-41 m gravel,
thin sand layers
Ground - 26 m: Bentonite

LEGEND:
Disturbed Sample
Undisturbed Sample
Headspace Analysis
Down Borehole Analysis
Groundwater Table
Perched Water Table

BOREHOLE LOG
JOB TITLE
LOCATION
CLIENT
REF No | JOB No

36 - 38; Karst, fracture, (nothing returned)
38 - 41 m bgl; Karst, nothing returned.

Appendix 4.f Drilling log of DG106



BOREHOLE NUMBER: DG 106 Page 1 of 1

DRILLING DATES:	DRILLING METHOD:
DRILLER:	BOREHOLE DIAMETER:
LOGGED BY:	SCREEN TYPE & DIAMETER:
CHECKED BY:	SCREEN SLOT SIZE:

BOREHOLE CONSTRUCTION | SAMPLE NUMBER | SAMPLE TYPE | SOIL VAPOUR (ppm) | GROUNDWATER | DEPTH (m) | GEOLOGY | DESCRIPTION | COMMENTS

0–0.6 m; Red brown clay. dry top soil.
0.6–3.5m; Red, sandy clay.
3.5–8m; Red sandy clay, with coarse gravels.
8–12.0m; Brown silty clay,
12.0 m; Limestone
12–12.3: Limestone
12.3–13.4 m: Red silty clay, sands
13.4–14.0 m; Red silty clay.
14 m; Limestone.
14–19 m; Dry, weathered limestone with yellow clay vein.
19–23 m; Weathered, yellow dusty,
23–25m: limestone.
25 m; weathered limestone, drill goes faster through 25–26 m.
28–33 m: Cavern, little material coming up, yellow. weathered limestone.

LOCATION / NOTES
25.2–31.2 m slotted, 24–31.2 m gravel, Thin sand layers, ground to 24m; Bentonite.

LEGEND
- Disturbed Sample
- Undisturbed Sample
- Headspace Analysis
- Down Borehole Analysis
- Groundwater Table
- Perched Water Table

BOREHOLE LOG
JOB TITLE
LOCATION
CLIENT
REF No | JOB No

Appendix 5 Label of wells (m AOD) and hydraulic gradients between two horizontal wells in subsoil, bedrock-interface and bedrock and the GWT (m AOD) (as of Jan, 2010) at all sites

Sites	Depth	Well ID	Ground Level Elevation (m AOD)	Screen Top Elevation (m AOD)	Screen Bottom Elevation (m AOD)	GWT depth (m AOD)	i ($\Delta h/\Delta l$)
JC	S	JC1A	59.459	56.459	54.959	58.26	0.006
JC	I	JC1B	59.459	50.459	48.459	58.19	0.013
JC	B	JC1C	59.459	43.459	41.459	57.94	0.012
JC	S	JC2A	57.160	55.160	53.160	55.87	0.030
JC	I	JC2B	57.160	48.160	45.160	53.21	0.002
JC	B	JC2C	57.160	39.160	37.160	53.17	0.003
JC	S	JC3A	42.983	36.983	35.483	42.34	0.032
JC	I	JC3B	42.983	31.983	29.983	42.25	0.032
JC	B	JC3C	42.983	21.983	19.983	39.69	0.024
JC	S	JC29	53.656	49.656	47.656	53.01	0.025
JC	I	JC30	53.675	43.675	41.675	52.94	0.025
JC	B	JC31	53.675	37.675	35.675	52.87	0.024
JC	S	JC32	32.012	28.012	26.012	30.31	0.020
JC	I	JC33	32.098	22.098	20.098	30.37	0.020
JC	B	JC34	32.098	16.098	14.098	30.83	0.021
River water table in JC is at approximately 29.56 mAOD							
SH	S	SH1A	98.510	96.510	94.510	97.38	0.025
SH	I	SH1B	98.510	89.010	87.010	93.40	0.005
SH	B	SH1C	98.510	78.510	76.510	93.53	0.006
SH	S	SH2A	92.755	89.755	87.755	91.48	0.022
SH	I	SH2B	92.785	88.285	86.285	92.24	0.007
SH	B	SH2C	92.785	79.785	77.785	92.22	0.007
SH	S	SH3A	91.814	88.814	86.814	91.54	0.003
SH	I	SH3B	91.861	84.361	82.361	91.60	0.003
SH	B	SH3C	91.861	73.861	71.861	91.59	0.003
Stream water table in SH is at approximately 91.53 mAOD							
OP	S	OP1A	56.114	52.114	50.114	54.06	0.003
OP	I	OP1B	56.114	47.614	45.614	53.83	0.008
OP	B	OP1C	56.114	32.214	26.214	52.59	0.006
OP	S	OP2A	54.089	50.089	48.089	52.86	0.003
OP	I	OP2B	54.191	45.691	43.691	49.98	0.008
OP	B	OP2C	54.191	36.191	30.191	49.95	0.006
River water table in OP is at approximately 51.59 mAOD							
DG	B	DG101	61.198	19.198	13.198	27.99	0.005
DG	B	DG102	61.609	5.609	5.609	28.21	0.007
DG	B	DG103	56.749	13.749	7.749	28.57	0.004
DG	B	DG104	54.319	17.319	11.319	30.74	0.021
DG	B	DG105	49.197	14.197	8.197	20.86	0.012
DG	B	DG106	46.132	20.932	14.932	20.12	0.008
River water table in DG is at approximately 18.49 mAOD							

Appendix 6 Estimation of dissolved gases in groundwater

The total gas concentration (TC) in the original water sample is calculated by first determining the gas concentration of the headspace, converting this to the partial pressure of the gas and then using this partial pressure to calculate the aqueous gas concentration which partitioned into the gas phase (C_{AH}), and aqueous phase concentration which remained in the aqueous phase (C_A). The total concentration (TC) in the aqueous phase is then:

$TC = C_{AH} + C_A$ (Eqn. 1)

where TC = total concentration of gas in the original aqueous sample C_{AH} = aqueous gas concentration in headspace after equilibrium; C_A = aqueous gas concentration in water after equilibrium. The concentration in the headspace is determined from calibration curves using standard gas samples. Parameters needed are the concentration of the gas component (Cg), Henry's law constant (H) for the gas, the temperature of the sample (T°C), the volume of the sample bottle (Vb), the headspace volume (Vh), and the molecular weight of the gaseous analytes (MW). For aqueous gas concentration in water after equilibrium, C_A: The concentration of the gas phase component is first determined using a calibration curve which was created by analyzing gas standards. The calibration curve provides the concentration of gas expressed in ppm based on volume of gas in total volume of sample. This concentration of gas is converted from ppm to the decimal equivalent of the volumetric concentration, Cg, by multiplying the ppm value by 10^{-6}. The partial pressure of the gas at atmospheric pressure, Pg, can be found by multiplying the gas volumetric concentration, Cg, by the atmospheric pressure.

In these calculations, total pressure, P_T is assumed to be equal to 1 atmosphere; therefore, Pg can be expressed with units of atm.

$Pg = Cg * P_T$ (Eqn. 2)

According to Henry's law, at equilibrium the mole fraction of the dissolved gas, x_g, can be determined from the partial pressure of the gas, P_g, and the Henry's law constant, H.

$x_g = P_g/H$ (Eqn. 3)

For these calculations, the Henry's law constant must be expressed in units of atm/mole fraction. The coefficients are applicable for sample temperatures between 14 and 40 °C.

Let n_g = mole of gas analyte and n_w = mole of water. Then the mole fraction of the dissolved gas can be expressed as

$x_g = n_g / (n_g + n_w)$

Rearranging

$n_g = x_g (n_g + n_w) = (x_g * n_g) + (x_g * n_w)$ (Eqn. 4)

If $n_g << n_w$, then $n_g = x_g * n_w$. (Eqn. 5)

Combining Eqn. 4 and 5

$n_g = n_w (P_g/H)$; (Eqn. 6)

and dividing each side by volume

$n_g /V = (n_w/V)*(P_g/H)$ (Eqn. 7)

Since the molar concentration of water, n_w /V, is 55.5 mol L^{-1}, then

$n_g / V = (55.5 \text{ mol } L^{-1}) (P_g/H)$ (Eqn. 8)

The saturation molar concentration of the gas component, C_A is defined as

$C_A = (n_g/V) (MW)$ (Eqn. 9)

where MW = molecular weight of the analyte, g mol^{-1}.

Substituting Eqn. 8 and 9 and converting to mg L^{-1}, the saturation molar concentration becomes gas concentration in the aqueous phase

$C_A = (55.5 \text{ mol } L^{-1}) * p_g/H * MW (g \text{ mol}^{-1}) * 10^3 \text{mg } g^{-1}$ (Eqn. 10)

where the final concentration is expressed in mg L^{-1}.

For the aqueous gas concentration in the headspace after equilibrium, C_{AH}:

gas, can be calculated at standard temperature by

$D = [MW / (22.4 \text{ L mol}^{-1})] * [273 \text{ K} / (T + 273 \text{ K})]$ (Eqn. 11)

where D = density (g L^{-1}) and T = sample temperature in °C. For the gas/water sample, the volume of the aqueous phase, V_a is the difference between the bottle volume, V_b and the headspace volume, V_h.

$V_a = V_b - V_h$ (Eqn. 12)

The volume of gas equilibrated into the headspace, A_h, can be determined from the volumetric concentration of the gas, C_g, and the volume of headspace, V_h.

$A_h = V_h * C_g$ (Eqn. 13)

The concentration, C_{AH}, of the gas component that was originally in the liquid phase but was then partitioned into the gas phase is

$C_{AH} = A_h/V_a$ (Eqn. 14)

Substituting Eqn. 4.20 and Eqn. 13 and 14; $C_{AH} = [V_h /(V_b - V_h)] * Cg$ and multiplying by the gas density expression, Eqn. 11, to convert from concentration units of mL of gas mL of water to mg of gas mL^{-1} of water, the concentration of gas in the water sample partitioned into the headspace, C_{AH}, becomes:

$C_{AH} = [(V_h /(V_b - V_h)]*C_g*(MW/22.4\ Lmol^{-1})*[273\ K/(T + 273K)]*10^3\ mg\ g^{-1}$ (Eqn. 15)

Then, combining Eqn. 10 and Eqn. 15

$TC = C_{AH} + C_A$

$TC = (55.5\ mol\ L^{-1})*\ p_g/H*\ MW\ (g\ mol^{-1})*10^3\ mg\ g^{-1} +$

$[(V_h /(V_b - V_h)] * C_g * (MW(g\ mol^{-1})/(22.4\ L\ mol^{-1})) * [273\ K / (T + 273\ K)] * 10^3\ mg\ g^{-1}$

The result will be in units of milligrams of gas L^{-1} of water. The partial pressures of CO_2 and CH_4 in equilibrated headspace and water were calculated using solubility of gases from Weiss (1974) for CO_2 and Wilhelm et al. (1977) for CH_4 at the recharge temperature as measured at the interface between the unsaturated zone and groundwater surface.

Appendix 7 EVALUATIONS OF HEADSPACE EQUILIBRATION METHODS FOR QUANTIFYING GROUNDWATER GHGs

Introduction

Measurement of dissolved gases in groundwater is increasingly becoming more common. Methane, N_2O and CO_2 are important GHGs that contribute to global warming (Ferron et al., 2007). Indirect N_2O emissions are now recognized as a quantitatively significant component of the total N_2O emission budget from agricultural activities (Mosier et al., 1998). Indirect N_2O emissions from drainage water can account for 50.3-67.3% of the above ground direct emissions (Minamikawa et al., 2010).

Dissolved CO_2 in river water is an important component of the terrestrial carbon cycle and an important pathway for CO_2 emissions to the atmosphere (Minamikawa et al., 2010). Groundwater in the UK was an important source of dissolved CO_2 in the rivers (Worrall and Lancaster, 2005). Stream flow in the Amazonian headwater catchment was predominantly derived from deeper flow paths containing water with high dissolved CO_2 concentrations (Johnson et al., 2006). High CH_4 concentrations in groundwater from the four major UK aquifers were observed where groundwater had the highest reductive potential (Gooddy and Darling, 2005). The concentration gradient of dissolved CH_4 between ambient air and water bodies will cause a substantial amount of CH_4 emissions from groundwater to the atmosphere (Sawamoto et al., 2003). Substantial amounts of CH_4 $\delta^{13}C$ (-72.1±6.8‰) and $\delta^{2}H$ (-297±17‰) from biogenic origin predominantly from CO_2 reduction from shallow groundwater wells in Alberta, Canada was reported by Cheung et al. (2010). Therefore, the concentrations of dissolved gases analysis in surface water, groundwater, drainage water, pore water etc. is important in evaluating biological activities within subsurface soils and sediments contaminated with NO_3^- and petroleum fuels, as well as evaluating the indirect sources and concentrations of major GHGs.

The headspace equilibration technique is a widely used method for extracting dissolved gases in water due to its simplicity, reliability and adaptability to routine analysis of samples (Kampbell et al., 1989). But this method has been used by numerous researchers with diverse ratios of *He*: water (v/v) and shaking times for equilibrating the dissolved gases between liquid and headspace. For example, Geistlinger et al. (2010) measured dissolved N_2O, CO_2 and CH_4 in groundwater with 1:1 headspace to water ratio and shaken

for 2-h. Hamilton and Ostrom (2007) extracted groundwater dissolved gases at a ratio (*He*: water) of 2:1 (v/v) and vigorously agitated for 5 min. Conversely, Lemon (1981) degassed water with headspace: water = 1:4 (v/v) and frequently shaken for several days. Reay et al. (2003) analysed dissolved gases in drainage water using an empty space: water ratio of 3.4:1 and vigorously shaken for 2 min followed by a 30 min standing period. However, Kellogg (2005) analysed groundwater for dissolved gases (N_2, N_2O, ^{15}N, $^{15}N_2O$, SF_6) using a headspace: water ratio of 6.5:1. In drainage water from lysimeters, Minamikawa et al. (2010) measured annual emissions of GHGs (N_2O, CO_2 and CH_4) using *He*: water ratio of 1:1 (v/v) and vigorously shaken for 1 min. This headspace equilibration technique with numerous ratios and shaking times raises the issue of the comparability of results. We hypothesise that headspace: water ratio, shaking times and standing period affect the extent of equilibration of gases dissolved in ground/surface water. The objective of this chapter was to (i) evaluate the effects of *He*: water ratio, shaking times and standing period on the extractions of GHGs and (ii) to examine if the treatment effects vary with the variations in existing gas concentrations in groundwater.

Groundwater sampling and analysis

Groundwater sampling was carried out in 3 monitoring wells (0.055 m ID; 2.0 m screen section) installed at 5 m bgl in a transect along groundwater flow path under intensively managed grazed grassland in Southeast Ireland (52°20'3" N, 6°27'27" W) shown in Figure 3.1 of Chapter 3. Each well was approximately 300 m apart with contrasting hydrogeochemical properties and partial pressures of GHGs which are summarized in Table 8.3. A total of 300 samples were collected using a bladder pump (Geotech Environmental Equipment, Inc., USA) following USEPA Region I Low Stress Purging and Sampling Procedures (USEPA, 1996). Samples were collected from the Teflon pump outlet tube (ID 0.006 m) at a rate of 100 ml min^{-1} so that withstanding of pressure does not cause any ebullition of dissolved gases. Water samples were collected in 160 ml glass serum bottles keeping the pump outlet tube at the bottom. At first similar volumes of water was overflowed and immediately sealed with butyl rubber septa and aluminium crimp caps (WHEATON, USA). No visible air bubbles were observed inside the bottle. All samples were submerged below water in a cool box, stored at 4°C and analysed within one week. For each well, samples were collected without labelling, treatments randomly assigned and then labelled to examine the effects on dissolved GHGs of headspace: water ratio, shaking time and standing time between headspace and water.

Treatments used

The experiment comprised 3 different types of treatments as below:

a. Five *He*: water ratios (v/v): R1 (0.75: 0.25), R2 (0.60: 0.40), R3 (0.50: 0.50), R4 (0.40: 0.60) and R5 (0.25: 0.75);
b. Five shaking times: S1 (0 min), S2 (1 min), S3 (5 min), S4 (10 min) and S5 (20 min); and
c. Four standing times: T1 (0 min), T2 (15 min), T3 (30 min) and T4 (60 min).

Samples were degassed using high purity *He* (BOC, Linde Group, Germany). The required headspace volume was augmented to 120, 96, 80, 64 and 40 ml by injecting *He* and replacing water simultaneously through the rubber septum of sealed serum bottle using a hypodermic needle and a polyvinyl syringe resulting in the *He*: water ratios of R1, R2, R3, R4 and R5. The samples were shaken on a gyrotory shaker (Model G-10, New Bruns- wick Scientific Co., USA) at 400 rpm for the required time as per the treatments levels S1-S5. Samples were then left undisturbed at room temperature (21°C) for the required times T1-T4. After equilibration, a headspace gas sample was collected in a 12 ml exetainer (Labco Ltd., Wycombe, UK) with an additional injection of 15 ml *He* using a PVC syringe and the dilution factor was taken into consideration during the calculation of dissolved gases. The N_2O, CO_2 and CH_4 were analysed by gas chromatography (CP-3800 GC, Varian, Inc. USA/CTC Analytics combi PAL Auto Sampler, Switzerland) equipped with an electron capture detector (ECD) using Ar as a carrier gas, a thermal conductivity detector (TCD) using Ar as a carrier gas, and a flame ionization detector (FID) using Ar as a carrier gas and H_2 to light the flame. The GC has a Porapak-Q column (80-100 MESH), 3.7 m x 1/8" x 2.0 mm. Calibration of the GC system was conducted using a minimum of three to a maximum of seven concentrations of each standard gas. The precision of analysis was satisfactory with coefficients of variation between analyses of 1.3%.

Estimating dissolved GHGs and hydrochemical analysis

The N_2O, CO_2 and CH_4 concentrations in water samples were estimated using Henry's law constant, the concentrations of the gas in the headspace, the bottle volume and the temperature of the water considered as recharging water temperature. The partial pressures of N_2O, CO_2 and CH_4 in the equilibrated headspace and water were calculated using solubility of gases from Weiss and Price (1984) for N_2O, Weiss (1974) for CO_2 and

Wilhelm et al. (1977) for CH_4 at the recharge temperature as measured at the interface between the unsaturated zone and groundwater surface.

Statistical analysis

As the emphasis in this study is on determination of optimum operating points, a regression analysis was used rather than a hypothesis testing approach like ANOVA. For regression on the experimental factors as continuous variables, the MIXED procedure (SAS, 2009) was used for a formal analysis of significant effects and response-surface analysis was used to examine the general effects of the treatment variables and to establish optimal operating points for them. The RSREG procedure (SAS, 2009) fits a quadratic response model and provides plots of response surfaces with estimates of optimal points in the treatment space. Residual checks were made and responses were transformed as required to ensure that the assumptions of the analysis (Normality and constant variance) were met.

Results of the effects of headspace to water ratio, shaking and standing time on N_2O extraction

The experiment was conducted in three hydrogeochemical contrasting wells as shown in Table 9.1. The details of the treatments used and experimental design is explained in the Chapter 4. Mean N_2O concentrations were 0.0317, 0.0263, 0.0212, 0.0208, and 0.0198 mg N L^{-1}, respectively in *He*: water ratios of R1, R2, R3, R4 and R5 (Figure 9.1). *He*: water ratio of 0.75: 0.25 (R1) showed significantly ($p<0.01$) higher N_2O concentration than R5 but was similar to R2, R3 and R4. Considering shaking duration, mean N_2O concentrations in 5 different shaking durations were 0.017, 0.027, 0.026, 0.027 and 0.023 mg N L^{-1} where no shaking (S1) showed significantly ($p<0.05$) lower than S2, S3, S4 and S5 but the later 4 were similar. Standing time did not appear to affect N_2O concentration significantly.

Table Hydrogeochemical properties of the wells sampled for the analyses of dissolved gases

Parameters	Well JC1A	Well JC1B	Well JC2A	Mean ± SE
N_2O (mg N L^{-1})	0.048	0.019	0.005	0.024 ±0.013
CO_2 (mg C L^{-1})	12.61	21.26	26.53	20.13 ±4.06
CH_4 (mg C L^{-1})	3.06	0.77	1.07	1.63 ±0.72
Ground water table (m)	2.27	2.29	1.97	2.17±0.10
K_{sat} (m d^{-1})	0.012	0.011	0.013	0.012 ±0.001
pH	6.84	6.78	6.45	6.69±0.12
Dissolved oxygen (mg L^{-1})	2.67	3.03	4.38	3.36 ±0.20
Redox potential (mV)	118	131	140	123 ±8.82
Dissolved organic C (mg L^{-1})	0.90	0.77	13.47	5.04±4.21
NO_3^--N (mg L^{-1})	2.55	4.62	2.73	3.10 ±0.78

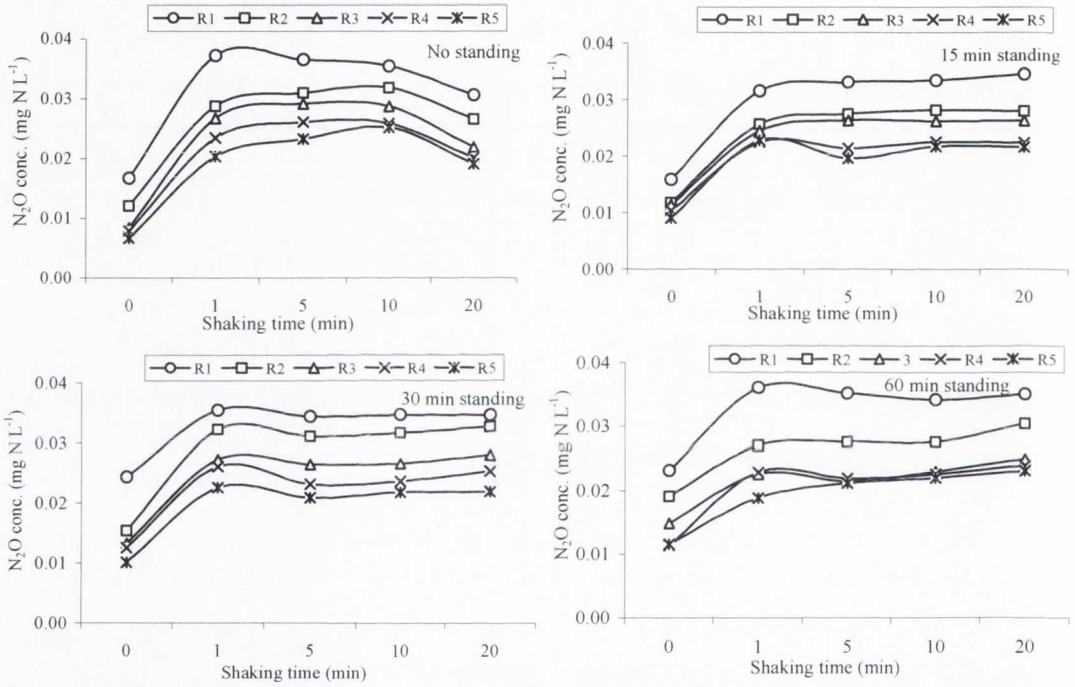


Figure N_2O concentrations in five different He: headspace ratios, shaking time and standing time

A formal stage of the analysis fitted the response surfaces using regression in Proc MIXED (SAS, 2009) to statistically evaluate the evidence for curvature in each treatment variable and then a graphical evaluation with Proc RSREG provided interpretation of the formal analysis. Ratio, shaking time and standing time were fitted as continuous variables and non-significant terms were removed until all remaining terms were significant or were contained in higher-order terms. Crossed terms and quadratic terms indicate curvature in the relationships. As the focus in the analysis is on selecting, if possible, an optimum operating point, graphical methods were used instead of examining the coefficients in detail. Plots were made of predicted values of the measurement variable, showing how it varied with the experimental factors. It was not practical to represent the relationships for

all three variables on one plot. One variable was held fixed, at a range of values, while the others varied continuously, producing a set of 3-dimensional plots.

For the N_2O results, one of the wells produced very low response values and these responses were removed from the analysis as the low variation within those responses allowed the detection of what appeared to be spurious patterns, quite different to the general behaviour across the rest of the data set. The range of the responses in the analysis was from 0.004 to 0.86 mg N L^{-1}. Residual checks for the N_2O results showed evidence of non-constant variance and a log transformation was used to compensate for this. The formal analysis of the regression relationships (Table 9.2) showed that there was curvature in the relationships with all the experimental factors. There was a significant quadratic term for each as well as crossed terms which allowed the slope of a linear relation with one variable to change smoothly as another variable changed. The terms showing interactions with wells provided evidence that the quadratic coefficients for standing time and shaking time varied from well to well. Examination of plots of the type illustrated in Figure 9.2 was used to interpret the results shown in Table 9.2.

Table Tests of effects from regression analysis of log-transformed N_2O results ($p < 0.5$ for inclusion in the final model)

Effect	Pr > F
Well	<.0001
Ratio	0.0019
ShakeTime	0.0404
ShakeTime*Well	<.0001
Ratio*Well	<.0001
StandingTime	0.0049
StandingTime*Well	<.0001
Ratio*StandingTime	0.0104
Ratio*Ratio	0.0234
ShakeTime*ShakeTime	0.0247
StandingTime*StandingTime	0.2679
Standingtime*Standingtime*Well	0.0041
ShakeTime*ShakeTime*Well	<.0001

The graphics indicated that there was a consistent optimum operating point for shaking time, approximately in the centre of the range examined. There was a good indication that the standing time was optimum or near-optimum at the high end of the range, but an optimum value for ratio was not covered by this data set. While there was significant curvature detected in this latter functional relationship, the response continued to increase

as the ratio approached the maximum value of 3. Determination of the optimum, based on this data set would require extrapolation. The RSREG procedure identified a maximum in the fitted surface and estimated it at ratio 4.4, shaking time 13 min and standing time 63 min, with only the shaking time covered by this data set. Plots were prepared for each well separately to check for effects of the interactions of the terms with wells. The relationship with shaking time was stable across wells while well 1 indicated a standing time optimum within the range in the data set.

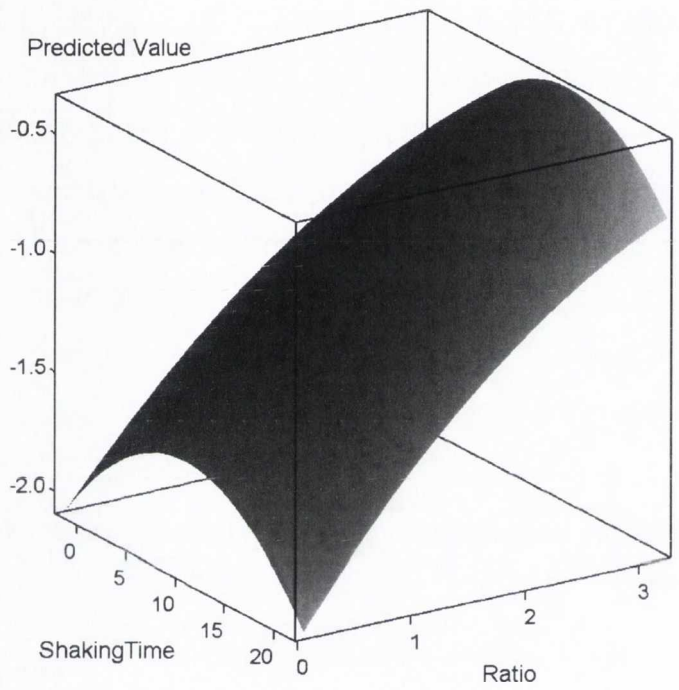


Figure Graphical representation of the fitted response surface for shaking time and ratio at a fixed value of 60 minutes for standing time. The predicted value refers to the log concentration of N_2O.

Results of the effects of headspace to water ratio, shaking and standing time on CH_4 extraction

Mean CH_4 concentrations in 5 different *He*: water ratios were 2.51, 1.89, 1.53, 1.31 and 0.92 μg C L^{-1} (Figure 9.3) where R1 showed significantly higher concentrations than R4 and R5 ($p<0.001$) and similar concentrations to R2 and R3 ($p>0.05$). Mean CH_4 concentrations in 5 shaking durations were 0.88, 1.47, 1.76, 1.89 and 2.15 μg C L^{-1}, respectively in no shaking (S1), 5 (S2), 10 (S3), 15 (S4) and 20 min (S5) shaking where S1 was significantly lower than S4 and S5 ($p<0.01$) but was similar to S2 and S3. The CH_4 concentrations were significantly affected ($p<0.001$) by standing times having mean values

of 0.87, 1.55, 1.60 and 2.15 mg L^{-1}, respectively in 0 (T1), 15 (T2), 30 (T3) and 60 (T4) min standing giving significantly higher concentrations in T4 than T1, T2 and T3 where the later three were statistically similar.

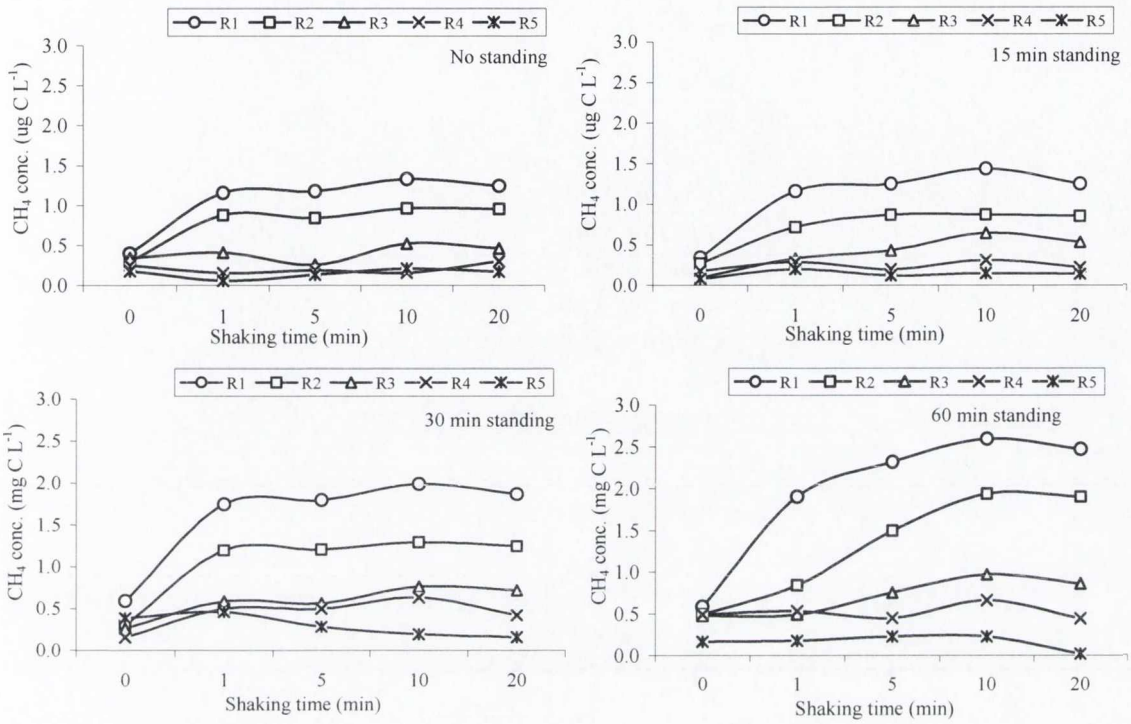


Figure CH_4 concentrations in five different He: headspace ratios, shaking time and standing time

CH_4 data (ranging from 0.003 to 9.097 mg C L^{-1} did not meet an assumption of Normal distribution and a square root transformation was found to be appropriate. The formal analysis in Table 9.3 shows quadratic terms for ratio and shaking time but not for standing time. Nor does standing time interact with either ratio or shaking time. There is a linear term only with evidence, from the interaction term involving wells that the slope varies between wells. Graphics confirmed this increasing trend across the range of standing times examined. An optimum value for standing time is not covered by this data set nor was there statistical evidence that an optimum existed. However, plots for standing time with curvature terms included did indicate that the trend in curvature was such that there would be an optimum at some greater value of standing time. Furthermore, while the data suggested that there was an optimum value of ratio near the high end of the range, it was not well defined and there was a linear trend that varied from well to well. An optimum for shaking time was better defined in the middle of the range. A maximum in the regression equations was determined at ratio 3.4, shaking time 13 min and standing time 108 min. The standing time estimate was determined by the fitting non-significant curvature terms to

underline that an optimum point was likely to be considerably outside the range of values in this study. It was noted that the estimated optimum ratio was close to the range of ratios examined.

Table Tests of effects from regression analysis of square root-transformed CH_4 results ($p < 0.5$ for inclusion in the final model)

Effect	Pr > F
Well	0.0079
Ratio	<.0001
Shaking Time	0.0007
Shaking Time*Well	<.0001
Ratio*Well	0.0176
Standing Time	<.0001
Standing Time*Well	<.0001
Ratio*Ratio	0.0087
Shaking Time*Shaking Time	0.0238

Results of the effects of headspace to water ratio, shaking and standing time on CO_2 extraction

Mean CO_2 concentrations of 23.27, 15.14, 12.15, 10.07 and 7.89 mg C L^{-1}, respectively in R1, R2, R3, R4 and R5 (Figure 9.4) were significantly affected by *He*: water ratios ($p<0.001$) providing higher concentrations in R1 than R2, R3, R4 and R5. Mean CO_2 in R2 was similar to R3 but was significantly higher ($p<0.001$) than R4 and R5 where later two (R4 and R5) were similar. The treatment R3 was significantly higher for CO_2 than R5. Shaking durations significantly affected CO_2 extractions ($p<0.001$) showing mean values of 9.41, 14.86, 14.04, 15.37 and 14.85 mg C L^{-1}, respectively in S1, S2, S3 , S4 and S5. Mean CO_2 concentrations in S1 was significantly lower than S2, S3, S4 and S5 where the later 4 were similar. No significant effect of standing time on CO_2 concentration was observed.

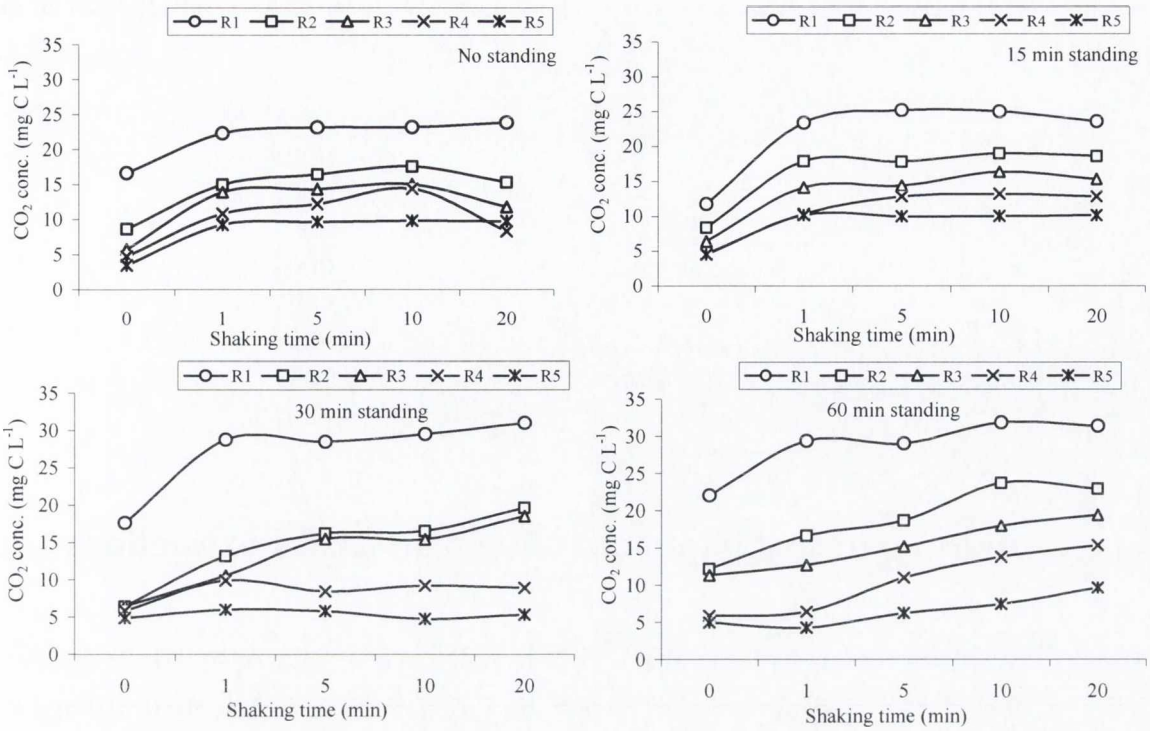


Figure CO_2 concentrations in five different He: headspace ratios, shaking time and standing time

Measurements for CO_2 varied from 0.19 to 54.39 mg C L^{-1}. For log CO_2, examination of the reduced model in Table 9.4 showed that there were significant quadratic terms for ratio and shaking time while standing time showed only a linear trend that varied from well to well, indicating possible optima for ratio and shaking time but no evidence of an optimum for standing time. Graphical representations indicated that, as for CH_4 and N_2O, there was a clear optimum shaking time in the middle of the range examined with an indication that there was little impact of standing time on the results (the magnitude of the slope in the linear effect was small). The ratio was found to be near an optimum at the top of its range but this was not well-defined. No maximum was found in the predicted response surface. When quadratic terms were fitted for standing time, a saddle point, where there is a maximum for one or more variables with a minimum for others, was estimated at ratio 3, shaking time 12 min and standing time 17 min. The standing time produced the minimum.

Table Tests of effects from regression analysis of log-transformed CO_2 results ($p < 0.5$ for inclusion in the final model)

Effect	*Pr > F*
Well	<.0001
Ratio	<.0001
Shaking Time	<.0001
*Ratio*Well*	<.0001
Standing Time	0.9827
*Standing Time*Well*	0.0053
*Ratio*Ratio*	0.0003
*Shaking Time*Shaking Time*	<.0001

Interpretation of treatment effects on GHGs extractions

Dissolved N_2O extraction in groundwater with *He*: water ratio 1:1 by Geistlinger et al. (2010) and 1.36:1 by von der Heide et al. (2008) were respectively 0.010 and 0.013 mg N L^{-1} with 120 min shaking in similar geochemical environments to this study (Table 9.5). Interestingly, their results were close to these results with *He*: water ratio 1:1 (0.012 mg N L^{-1}) which increased to 0.032 mg N L^{-1} at a ratio of 0.75:0.25. Similarly, Lemon (1981), Ferron et al. (2007) and Reay et al. (2003) measured dissolved N_2O concentrations in surface waters with *He*: water ratios, respectively 1:1, 1.5:1 and 3.4:1 giving 0.0003, 0.0002 and 0.0020 mg N L^{-1}. These results indicate that headspace volume should be 3 times higher than water volume to completely equilibrate N_2O dissolved in water, otherwise the extracted concentration can be underestimated. This outcome is in broad agreement with the results in this paper where a ratio of at least 3:1 is seen to be required. Similarly, poor results from other *He*: water ratios from the current work confirmed that *He*: water ratio less than 0.60:0.40 provides very poor equilibration between gases and liquid and hence results in low-biased measured concentrations of N_2O. It is clear that shaking time is required for degassing of dissolved N_2O and that an optimum is approximately 13 minutes.

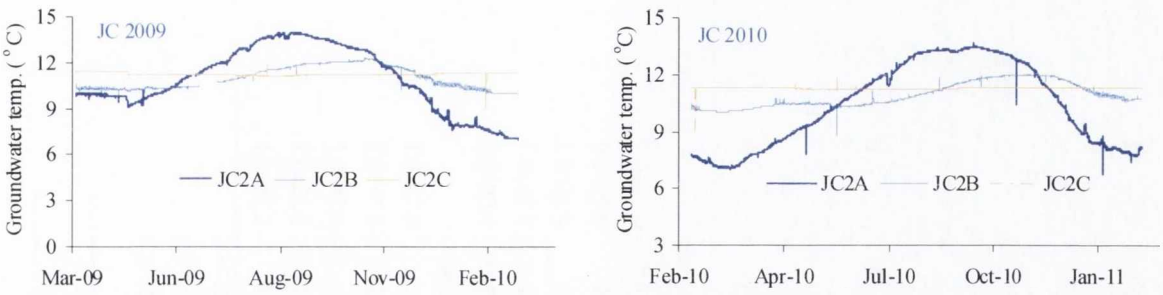
The CO_2 concentration (14.4 mg C L^{-1}) measured by von der Heide et al. (2008) with 1.36:1 ratio was similar to this study (12.3 mg C L^{-1}) at a similar ratio but at the higher ratio 0.75:0.25 in this study CO_2 doubled to 26.8 mg C L^{-1}. To obtain complete equilibration in CH_4 concentrations it is clear that a *He*: water ratio of at least 0.75: 0.25 is essential because lower *He*: water ratio definitely underestimated CH_4 concentration. It was clear from the results, that the CH_4 equilibration required at least 10 minutes shaking

but shaking times above that did not significantly increase CH_4 concentration and that the estimate of 13 minutes from the RSREG procedure was entirely consistent with this. Minamikawa et al. (2010) measured CH_4 concentration (0.36 ug C L^{-1}) with a 1:1 ratio in drainage water from a similar soil type which was close to this result (0.40 ug C L^{-1}) with same ratio. However, in this study CH_4 concentration more than doubled to 98 ug C L^{-1} at with the same ratio but with a shaking time 10 min and a standing time 1 h. The evidence that relationships varied from well to well (interactions terms with well in the analysis) were evaluated using plots of the predicted surfaces for individual wells showed and this showed nothing incompatible with the overall conclusions but sufficient variation to warrant an extended study for verification of the results. This study showed that headspace volume, shaking time and standing time are critical factors in the estimation of dissolved greenhouse gases in water under a range of existing geochemical environments. Only the shaking time was found to have a consistent optimum operating point in this study. The study also provided indications that the optima for the other factors are greater than the highest values covered. The standing time has the most variable impact on the results. It appears to vary substantially between gases and between wells.

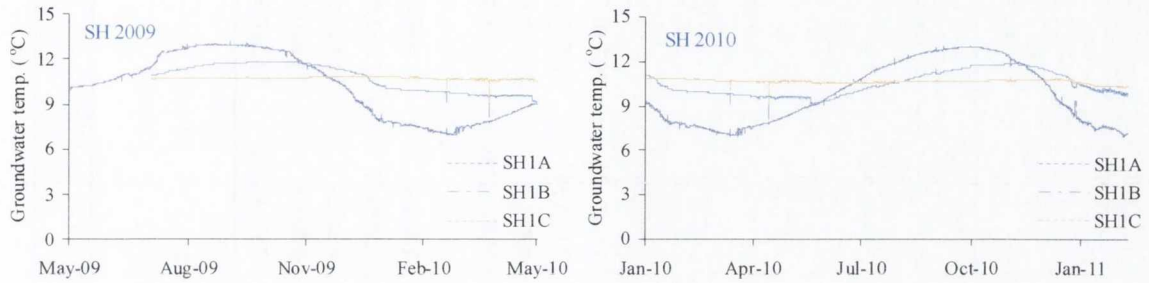
Table Comparisons of headspace extraction methods used for dissolved GHGs by different researchers

Authors	Source of water	*He*: water	Shaking time (min)	Standing time (min)	N_2O ($mgNL^{-1}$)	CO_2 ($mgCL^{-1}$)	CH_4 ($ugCL^{-1}$)
Geistlinger et al. (2010)	groundwater	1:1	120		0.010		
von der Heide et al. (2008)	groundwater	1.36:1	120		0.013	14.4	
Clough et al. (2007)	groundwater	7:1	1		0.004 (no nitrate)		5
Reay et al. (2003)	Surface water (Drain from arable land)	3.4:1	2	30	0.0020		
Ferron et al. (2007)	Tidal water in fish farm	1.5:1	vigorous		0.0002	0.180	0.07
Lemon (1981)	Surface water (Lake)	1:5	several days		0.0003		
Minamikawa et al. (2010)	Agricultural drainage water (Rice)	1:1	1		0.079	16.5	0.36
Xu et al. (2009)	Temp. oldforest soil soln.	1:1	5	5	20-99 ng ml^{-1}	3.5-18.3 µg ml^{-1}	

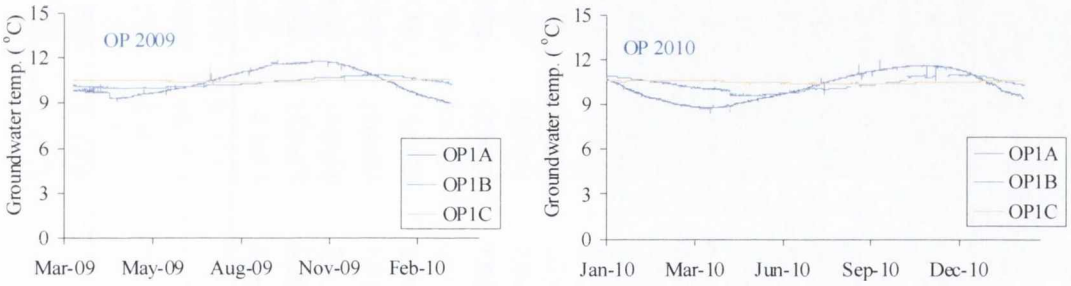
Appendix 8.a Groundwater temperature changes in every 30 min at JC site



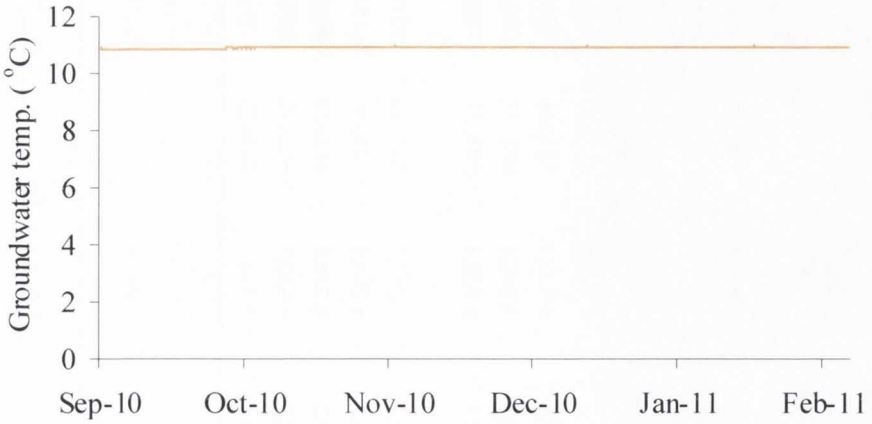
Appendix 8.b Groundwater temperature changes in every 30 min at SH site



Appendix 8.c Groundwater temperature changes in every 30 min at OP site



Appendix 8.d Groundwater temperature changes in every 30 min at DG site



Appendix 9.a Mean hydrogeochemical properties of groundwater at JC during 2009-2010

Well ID	GWT (m bgl)*	PH	EC ($\mu S\ cm^{-1}$)	Eh (mV)	DO ($mg\ L^{-1}$)	DOC ($mg\ L^{-1}$)	CO_2 ($mg\ C\ L^{-1}$)	CH_4 ($\mu g\ C\ L^{-1}$)	Cl^- ($mg\ L^{-1}$)	SO_4^{2-} ($mg\ L^{-1}$)	S^{2-} ($mg\ L^{-1}$)
Subsoil											
JC1A	2.0±0.2	6.8±0.1	561±19	160±11	1.8±0.4	1.3±0.2	34.9±2.2	2.65±0.77	37.6±1.3	28.8±0.8	0.33±0.1
JC2A	2.0±0.2	6.3±0.1	342±17	148±17	4.2±0.6	15.9±1.4	76.7±6.6	2.08±0.60	34.1±1.9	26.6±2.4	0.19±0.1
JC3A	1.5±0.3	6.8±0.1	501±16	69±19	1.9±0.2	1.0±0.2	41.5±2.1	3.59±1.0	29.9±0.5	16.1±0.4	0.32±0.1
JC29	1.1±0.1	7.1±0.2	505±20	97±16	2.8±0.6	1.0±0.2	23.9±2.7	42.07±12	32.2±0.9	14.2±0.2	0.32±0.1
JC32	1.8±0.1	7.0±0.1	510±14	63±23	1.6±0.4	0.8±0.1	22.3±1.2	66.26±19	27.0±0.8	12.7±0.3	0.15±0.1
Interface											
JC1B	2.1±0.2	6.7±0.1	462±9	161±12	1.8±0.7	1.0±0.3	44.1±3.0	1.7±0.41	27.7±0.9	12.9±0.3	0.31±0.1
JC2B	4.6±0.2	6.8±0.1	582±20	-69±30	0.5±0.1	8.1±0.3	36.1±1.7	149. 2±21	40.7±0.7	30.3±1.4	0.06±0.0
JC3B	2.0±0.3	6.9±0.1	531±14	92±14	2.6±0.5	0.8±0.1	40.6±2.4	2.6±0.50	32.7±1.1	22.3±2.0	0.36±0.1
JC30	1.2±0.1	6.9±0.1	473±11	102±17	1.9±0.7	0.9±0.2	27.6±2.5	8.7±3.1	34.2±0.9	17.8±1.1	0.26±0.1
JC33	1.9±0.1	6.9±0.1	503±21	-32±22	0.5±0.1	1.2±0.2	28.9±4.2	2894 ±1386	27.3±1.5	11.5±0.7	0.09±0.0
Bedrock											
JC1C	2.2±0.2	6.7±0.1	437±10	151±11	2.1±0.3	0.5±0.3	31.7±1.2	1.6±0.4	31.5±0.6	14.1±0.2	0.21±0.1
JC2C	4.7±0.2	6.8±0.1	572±16	-45±13	0.6±0.1	9.8±0.5	37.7±2.0	158.9±15	41.2±1.0	29.8±1.2	0.26±0.1
JC3C	4.1±0.2	6.7±0.1	413±22	65±22	1.5±0.3	0.6±0.1	43.9±1.8	4.6±1.3	31.6±0.5	7.9±0.8	0.28±0.1
JC31	1.3±0.1	6.9±0.1	514±14	112±14	1.3±0.4	0.7±0.2	24.3±1.9	4.7±1.5	33.8±0.7	16.0±1.0	0.22±0.1
JC34	1.6±0.1	7.0±0.1	441±23	-7±16	0.5±0.1	0.7±0.4	18.1±4.0	352. 4±131	31.2±0.9	11.0±0.4	0.20±0.1

Appendix 9.b Mean hydrogeochemical properties of groundwater at SH during 2009-2010

Well ID	GWT (m bgl)*	PH	EC ($\mu S\ cm^{-1}$)	Eh (mV)	DO ($mg\ L^{-1}$)	DOC ($mg\ L^{-1}$)	CO_2 ($mg\ C\ L^{-1}$)	CH_4 ($\mu g\ C\ L^{-1}$)	Cl^- ($mg\ L^{-1}$)	SO_4^{2-} ($mg\ L^{-1}$)	S^{2-} ($mg\ L^{-1}$)
Subsoil											
SH1A	1.2±0.1	6.8±0.1	648±22	129±18	1.31±0.2	1.93±0.2	61.2±6.3	1.8±0.5	19.5±0.6	13.5±0.7	0.25±0.1
SH2A	1.4±0.1	7.2±0.1	469±21	-2±18	0.68±0.1	1.58±0.6	9.8±0.7	44.5±13	21.6±0.5	16.5±2.0	0.14±0.1
SH3A	0.5±0.1	7.2±0.1	496±22	-78±27	0.81±0.1	1.13±0.2	12.2±1.5	46.8±14	27.2±2.5	12.6±1.3	0.24±0.1
Interface											
SH1B	5.3±0.1	6.9±0.1	620±20	125±16	2.70±0.3	2.20±0.2	47.4±6.1	16.6±18	15.4±0.5	10.3±0.5	0.15±0.7
SH2B	0.6±0.1	7.4±0.1	439±12	69±19	0.60±-.1	0.53±0.1	13.9±4.0	5.2±1.8	23.1±0.8	20.3±0.9	0.30±0.9
SH3B	0.3±0.1	7.4±0.1	459±13	80±26	0.52±0.1	0.66±0.2	14.3±3.4	18.3±16	19.9±0.7	9.2±0.7	0.17±1.5
Bedrock											
SH1C	5.1±0.1	6.9±0.1	619±20	123±10	4.61±0.5	1.71±0.2	45.2±1.9	8.2±6.8	17.3±0.8	14.1±2.6	0.07±0.0
SH2C	0.6±0.1	7.5±0.1	444±13	-1±25	0.46±0.1	0.90±0.5	10.6±3.0	4.8±1.0	22.4±0.9	24.3±5.9	0.27±0.1
SH3C	0.4±0.1	7.4±0.1	478±14	7±19	0.75±0.2	1.02±0.2	12.4±3.1	142±91	30.7±3.6	19.4±3.1	0.10±0.0

Appendix 9.c Mean hydrogeochemical properties of groundwater at OP during 2009-2010

Well ID	GWT (m bgl)*	PH	EC ($\mu S\ cm^{-1}$)	Eh (mV)	DO ($mg\ L^{-1}$)	DOC ($mg\ L^{-1}$)	CO_2 ($mg\ C\ L^{-1}$)	CH_4 ($\mu g\ C\ L^{-1}$)	Cl^- ($mg\ L^{-1}$)	SO_4^{2-} ($mg\ L^{-1}$)	S^{2-} ($mg\ L^{-1}$)
Subsoil											
OP1A	4.0±0.4	7.3±0.1	472±35	176±21	9.29±0.4	0.97±0.2	16.5±2.0	1.4±0.4	8.9±0.7	22.5±1.5	0.16±0.1
OP2A	1.3±0.1	7.5±0.1	509±34	131±16	3.32±0.8	1.24±0.5	9.1±1.2	19.5±5.6	22.2±1.2	13.6±0.8	0.18±0.1
Interface											
OP1B	4.2±0.4	7.3±0.1	474±15	167±19	9.65±0.5	0.88±0.2	13.7±1.8	1.3±0.3	13.2±0.8	14.9±1.0	0.29±0.1
OP2B	5.0±0.2	9.9±0.5	475±63	68±25	2.73±0.5	0.79±0.1	5.5±3.3	7.0±7.0	21.5±0.9	27.9±1.4	0.18±0.0
Bedrock											
OP1C	5.1±0.3	7.4±0.1	468±22	155±18	6.28±0.8	0.71±0.1	8.9±1.4	1.7±0.4	19.3±1.1	17.8±1.2	0.25±0.1
OP2C	5.2±0.3	7.7±0.3	489±16	151±11	3.32±0.7	0.58±0.1	14.7±2.7	2.4±1.0	21.3±0.5	23.5±0.8	0.10±0.0

Appendix 9.d Mean hydrogeochemical properties of groundwater at DG during 2009-2010

Well ID	GWT (m bgl)*	PH	EC ($\mu S\ cm^{-1}$)	Eh (mV)	DO ($mg\ L^{-1}$)	DOC ($mg\ L^{-1}$)	CO_2 ($mg\ C\ L^{-1}$)	CH_4 ($\mu g\ C\ L^{-1}$)	Cl^- ($mg\ L^{-1}$)	SO_4^{2-} ($mg\ L^{-1}$)	S^{2-} ($\mu g\ L^{-1}$)
Bedrcok											
DG101	32.5±1.0	7.0±0.1	625±41	172±21	8.16±0.8	0.99±0.2	35.7±3.5	1.3±0.3	18.6±2.6	22.5±1.3	0.19±0.0
DG102	33.5±1.0	6.9±0.1	574±15	178±21	9.17±0.6	1.04±0.2	33.5±2.7	1.1±0.2	8.7±0.3	13.6±1.0	0.24±0.1
DG103	28.3±0.7	6.9±0.1	593±23	169±20	9.33±0.7	0.79±0.1	35.1±2.7	1.3±0.3	11.9±0.6	14.9±0.9	0.19±0.1
DG104	24.1±0.4	6.8±0.1	712±20	186±20	8.30±0.7	1.12±0.1	40.9±3.6	1.5±0.3	26.9±1.7	27.9±1.9	0.12±0.1
DG105	29.2±0.4	6.9±0.1	579±24	174±21	8.59±0.7	0.76±0.1	23.4±1.5	1.3±0.3	21.8±0.5	17.8±0.7	0.05±0.0
DG106	26.4±0.2	6.9±0.1	682±32	177±19	8.73±0.7	0.82±0.1	30.1±3.0	1.3±0.3	25.4±0.7	23.5±1.0	0.06±0.0

Appendix 10.a Mean concentrations of basic elements and metals in groundwater at JC during 2009-2010

Well ID	Na ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	Ca ($mg\ L^{-1}$)	Mg ($mg\ L^{-1}$)	Total Fe ($\mu g\ L^{-1}$)	Fe (II) ($\mu g\ L^{-1}$)	Total Mn ($\mu g\ L^{-1}$)	Mn (II) ($\mu g\ L^{-1}$)	Zn ($\mu g\ L^{-1}$)	Cu ($\mu g\ L^{-1}$)	Cd ($\mu g\ L^{-1}$)	Cr ($\mu g\ L^{-1}$)	Ni ($\mu g\ L^{-1}$)	Pb ($\mu g\ L^{-1}$)
Subsoil														
JC1A	16.7±0.6	0.9±0.2	95.6±5	18.6±1.3	373.8±179	31.3±15	111.1±16	17.0±16	3.0±1.3	2.8±0.7	0.7±0.3	3.0±0.6	4.6±1.2	7.6±2.6
JC2A	16.8±1.1	20.1±3.2	47.4±4	14.3±0.9	643.5±300	82.6±16	73.4±20	35.0±20	3.8±1.0	5.8±1.0	0.4±0.1	2.5±0.3	4.3±0.9	8.1±2.8
JC3A	17.7±1.1	1.6±0.3	97.3±4	17.9±0.7	1196±435	15.9±6	300.9±7	16.5±2	2.0±1.0	4.0±1.1	1.0±0.6	3.2±1.2	3.8±0.8	5.3±1.7
JC29	21.9±1.1	2.8±0.5	91.2±4	18.0±0.5	6.9±2.6	0.8±0.4	836.9±351	92.5±35	1.7±0.9	3.0±0.6	0.4±0.1	1.4±0.4	2.5±0.7	6.4±2.5
JC32	17.9±0.8	2.0±0.2	94.6±3	20.7±0.8	632.0±294	9.9±5	42.4±20	11.2±7	1.4±0.6	5.2±1.3	0.5±0.1	1.9±0.4	2.0±0.7	9.4±3.0
Interface														
JC1B	15.9±0.5	0.6±0.1	88.4±3	17.2±83	175.6±1	0.5±0.3	5.9±1	5.0±2	1.8±0.9	2.0±0.4	0.5±0.1	1.7±0.4	3.1±0.8	5.3±2.1
JC2B	18.7±1.0	6.2±2.6	103±8	15.6±605	1221±0.7	189.7±84	562.9±34	331.1±83	2.7±1.2	5.8±0.4	0.5±0.2	2.6±0.4	5.2±1.5	8.6±2.4
JC3B	25.5±2.4	1.8±0.2	92.9±4	18.8±65	154.4±0.9	16.8±8	55.8±56	3.±0.8	3.6±2.0	5.5±1.4	1.6±0.7	3.7±1.4	3.9±1.0	7.5±2.6
JC30	21.7±0.8	2.5±0.4	84.2±2	19.9±3	6.6±0.7	4.3±2	131.0±57	7.0±6	1.5±0.6	3.3±0.4	0.5±0.1	1.4±0,4	2.1±0.7	8.0±2.5
JC33	23.4±2.2	3.4±0.4	85.2±3	18.9±36	88.5±0.7	28.1±5	389.7±55	144.2±74	0.2±0.1	3.1±0.5	0.4±0.1	1.4±0.5	2.8±0.9	7.7±1.9
Bedrock														
JC1C	21.3±1.0	1.0±0.1	77.1±2	17.3±0.6	112.1±51	0.7±0.2	20.2±8	3.1±1.3	0.5±0.2	1.8±0.4	0.6±0.2	1.5±0.3	3.7±1.1	7.6±2.6
JC2C	28.7±2.1	3.4±0.4	103±3	15.1±0.6	2334±1020	58.4±21	1433.7±29	863.7±180	11.5±9.4	5.9±1.6	0.6±0.2	2.3±0.3	5.6±1.0	7.3±2.0
JC3C	28.4±1.5	2.4±0.2	51.3±5	25.1±1.0	187±140	0.8±0.3	750.6±11	538.9±79	6.7±1.5	2.6±0.8	0.6±0.2	1.4±0.4	1.8±0.6	7.6±3.1
JC31	22.9±0.9	2.5±0.7	92.4±5	20.4±0.8	8.1±4	0.7±0.3	35.7±9	11.0±9	1.0±0.4	3.1±0.6	0.5±0.1	1.7±0.4	2.0±0.5	5.1±2.1
JC34	22.0±1.7	4.1±0.5	73.2±4	20.6±1.4	29.1±14	19.6±4	107.8±37	40.3±21	1.7±0.9	3.8±1.2	0.9±0.5	1.4±0.5	1.4±0.6	7.9±2.6

Appendix 10.b Mean concentrations of basic elements and metals in groundwater at SH during 2009-2010

Well ID	Na ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	Ca ($mg\ L^{-1}$)	Mg ($mg\ L^{-1}$)	Total Fe ($\mu g\ L^{-1}$)	Fe (II) ($\mu g\ L^{-1}$)	Total Mn ($\mu g\ L^{-1}$)	Mn (II) ($\mu g\ L^{-1}$)	Zn ($\mu g\ L^{-1}$)	Cu ($\mu g\ L^{-1}$)	Cd ($\mu g\ L^{-1}$)	Cr ($\mu g\ L^{-1}$)	Ni ($\mu g\ L^{-1}$)	Pb ($\mu g\ L^{-1}$)
Subsoil														
SH1A	18.7±1.4	7.7±0.7	113±6	15.8±0.9	370±116	6.7±1.8	19.6±9.1	10.8±3.3	3.3±1.7	5.3±1.3	1.4±0.2	2.5±0.7	2.2±0.8	6.8±1.8
SH2A	14.5±0.8	4.6±0.7	61.8±9	23.9±1.9	16.7±349	40.6±15	133.1±20	30.0±23	1.5±1.2	1.4±0.4	0.4±0.2	1.6±0.3	2.0±0.8	6.0±1.3
SH3A	19.5±0.9	2.4±0.4	69.7±3	25.9±0.7	64.2±135	42.1±7.8	269.1±44	73.0±28	2.5±2.1	1.8±0.6	0.7±0.2	1.4±0.3	1.3±0.4	3.2±1.0
Interface														
SH1B	15.9±0.5	8.9±0.6	111±5	15.0±0.3	176±0.3	12.5±2.2	6.8±1	3.6±2	1.5±1.1	3.8±1.1	0.8±0.3	1.4±0.4	2.3±0.7	8.6±2.9
SH2B	17.6±1.0	2.3±0.3	68.1±1	27.1±0.8	669±0.8	34.8±9.7	149.6±26	55.7±20	4.5±2.8	2.3±0.7	0.3±0.1	1.8±0.6	2.2±1.2	9.9±1.9
SH3B	30.7±1.4	2.3±0.3	66.7±2	23.3±0.7	210±0.7	40.7±13	386.4±91	59.5±22	2.3±1.6	2.7±0.8	0.5±0.2	1.0±0.3	2.3±1.1	6.9±2.1
Bedrock														
SH1C	16.1±1.4	8.4±0.4	109±3	15.6±0.6	260±143	16.6±7.6	8.8±5	2.7±1	1.6±1.1	3.4±1.1	0.4±0.1	1.6±0.4	1.3±0.5	7.4±1.6
SH2C	31.0±2.1	2.9±0.5	61.8±2	22.7±1.0	414±216	26.3±9.0	76.5±24	42.0±17	2.0±0.7	2.3±0.7	0.3±0.1	1.2±0.4	1.8±0.7	9.2±2.0
SH3C	46.3±4.8	2.9±0.4	64.5±2	22.5±0.7	225±121	13.7±1.4	139.7±34	29.0±11	0.4±0.6	2.3±0.6	0.5±0.1	1.0±0.2	2.7±1.0	6.3±2.0

Appendix 10.c Mean concentrations of basic elements and metals in groundwater at OP during 2009-2010

Well ID	Na ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	Ca ($mg\ L^{-1}$)	Mg ($mg\ L^{-1}$)	Total Fe ($\mu g\ L^{-1}$)	Fe (II) ($\mu g\ L^{-1}$)	Total Mn ($\mu g\ L^{-1}$)	Mn (II) ($\mu g\ L^{-1}$)	Zn ($\mu g\ L^{-1}$)	Cu ($\mu g\ L^{-1}$)	Cd ($\mu g\ L^{-1}$)	Cr ($\mu g\ L^{-1}$)	Ni ($\mu g\ L^{-1}$)	Pb ($\mu g\ L^{-1}$)
Subsoil														
SH1A	10.6±3.1	1.2±0.3	85.9±4.3	16.5±1.0	171±107	1.0±0.4	11.0±4.6	10.0±0.8	1.0±0.6	2.8±0.9	0.9±0.4	2.3±0.7	2.6±0.8	6.6±2.3
SH2A	9.3±1.6	3.0±0.7	83.6±4.9	12.9±1.0	7.7±4	1.8±0.5	1.9±0.5	1.0±0.9	0.4±0.2	0.8±0.2	0.8±0.3	1.9±1.1	3.4±0.9	4.3±1.6
Interface														
SH1B	9.3±0.3	1.4±0.6	89.8±3.9	11.5±0.6	69.4±43	1.9±0.6	5.2±2.1	0.5±0.3	2.6±1.8	2.8±1.7	0.3±.0.1	2.0±0.4	2.2±1.0	2.3±1.8
SH2B	15.5±3.5	4.2±1.1	60.6±7.4	8.6±1.1	85.3±48	1.6±0.4	26.1±14	0.0±0.0	1.3±0.6	1.5±0.5	0.5±0.2	2.6±1.7	4.5±1.3	2.9±1.2
Bedrock														
SH1C	12.6±2.1	2.1±0.0.4	87.6±4.0	14.1±0.8	40.4±20	0.5±0.3	2.0±0.7	0.0±0.0	1.0±0.8	2.2±0.8	2.1±1.0	3.9±1.1	4.7±2.5	6.1±3.2
SH2C	14.7±1.9	1.9±0.2	85.0±7.0	13.9±1.3	92.8±49	0.5±0.3	4.1±1.7	3.4±1.7	0.8±0.5	1.2±0.5	0.5±1.2	1.9±1.0	4.3±1.5	4.7±1.7

Appendix 10.d Mean concentrations of basic elements and metals in groundwater at DG during 2009-2010

Well ID	Na ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	Ca ($mg\ L^{-1}$)	Mg ($mg\ L^{-1}$)	Total Fe ($\mu g\ L^{-1}$)	Fe (II) ($\mu g\ L^{-1}$)	Total Mn ($\mu g\ L^{-1}$)	Mn (II) ($\mu g\ L^{-1}$)	Zn ($\mu g\ L^{-1}$)	Cu ($\mu g\ L^{-1}$)	Cd ($\mu g\ L^{-1}$)	Cr ($\mu g\ L^{-1}$)	Ni ($\mu g\ L^{-1}$)	Pb ($\mu g\ L^{-1}$)
Bedrcok														
DG101	10.6±0.5	2.4±0.4	1134±5.8	10.1±0.8	50.2±39	9.9±2.0	3.0±1.7	0.0±0.0	2.3±1.0	2.0±0.7	0.3±0.1	2.3±0.5	4.4±2.1	6.1±2.5
DG102	9.3±0.6	1.6±0.4	104±4.4	12.1±0.5	6.7±3	0.8±0.3	4.5±1.4	2.2±1.8	1.9±1.1	1.8±0.6	0.3±0.2	1.9±0.4	3.3±0.8	6.9±1.9
DG103	9.3±0.5	1.2±0.3	107±5.2	8.9±0.4	30.6±16	0.2±0.1	1.7±0.8	0.4±0.2	1.2±0.6	1.6±0.5	0.4±0.1	2.0±0.3	3.0±0.9	7.1±2.3
DG104	15.5±1.2	7.0±0.7	124±6.7	10.7±0.6	8.2±3	1.7±0.5	3.1±1.5	0.9±0.2	5.7±4.2	1.8±0.5	0.5±0.2	2.6±0.8	3.7±0.7	8.5±2.1
DG105	12.6±0.8	5.5±0.7	111±4.6	7.6±0.6	65.7±49	40.0±12	10.3±2.1	5.3±0.9	6.7±3.0	4.7±1.8	2.0±0.7	3.9±1.3	3.4±1.1	8.9±3.8
DG106	14.7±1.0	5.8±0.4	120±6.9	8.5±0.6	131.9±81	7.0±2.3	14.1±6.2	2.1±1.2	1.4±0.6	1.6±0.4	0.8±0.4	1.9±0.4	2.0±0.6	5.3±2.3

Appendix 11.a Mean dissolved N species and dissolved CO_2 and CH_4 gases in groundwater at JC during 2009-2010

Well ID	N_2O-N (mg N L^{-1})	Excess N_2-N (mg N L^{-1})	NO_3^--N (mg N L^{-1})	Total N Loadings (mg N L^{-1})	NO_2^- (mg N L^{-1})	NH_4^+ (mg N L^{-1})	DON (mg N L^{-1})	RP*	N_2O-N/NO_3^--N	N_2O/total N loadings
Subsoil										
JC1A	0.083±0.012	2.73±0.25	4.42±0.69	7.2±0.62	0.03±0.01	0.03±0.01	0.18±0.07	0.43±0.04	0.0241±0.0047	0.0119±0.0013
JC2A	0.029±0.008	0.61±0.13	2.90±0.55	3.5±0.61	0.00±0.00	0.05±0.01	0.82±0.13	0.23±0.05	0.0141±0.0040	0.0099±0.0029
JC3A	0.044±0.004	1.90±0.05	7.71±0.40	9.7±0.50	0.01±0.02	0.02±0.01	0.51±0.28	0.21±0.02	0.0058±0.0006	0.0046±0.0005
JC29	0.015±0.005	1.74±0.28	3.23±0.35	5.0±0.30	0.02±0.01	0.12±0.10	0.25±0.12	0.36±0.05	0.0066±0.0012	0.0033±0.0012
JC32	0.017±0.008	1.63±0.18	2.20±0.21	3.8±0.15	0.01±0.01	0.06±0.03	0.19±0.07	0.43±0.04	0.0114±0.0081	0.0047±0.0024
Interface				0.						
JC1B	0.032±0.007	1.57±0.14	5.68±0.31	7.3±0.22	0.00±0.00	0.02±0.01	0.19±0.08	0.22±0.02	0.0055±0.0010	0.0043±0.0008
JC2B	0.006±0.002	6.51±0.39	0.13±0.05	6.5±0.37	0.00±0.00	0.30±0.04	0.37±0.06	0.98±0.01	0.0418±0.0037	0.0011±0.0004
JC3B	0.039±0.006	1.39±0.16	8.11±0.37	9.5±0.58	0.00±0.00	0.02±0.01	0.64±0.38	0.15±0.02	0.0049±0.0007	0.0041±0.0006
JC30	0.023±0.010	1.68±0.17	4.83±0.28	6.5±0.51	0.01±0.00	0.03±0.01	0.33±0.13	0.26±0.03	0.0049±0.0022	0.0036±0.0016
JC33	0.005±0.002	1.95±0.14	0.30±0.14	2.2±0.22	0.02±0.01	0.75±0.41	0.17±0.06	0.88±0.05	0.0268±0.0108	0.0024±0.0009
Bedrock										
JC1C	0.032±0.004	1.52±0.13	6.92±0.13	8.5±0.20	0.00±0.00	0.02±0.01	0.19±0.11	0.18±0.01	0.0046±0.0005	0.0038±0.0005
JC2C	0.010±0.003	5.30±0.37	0.24±0.09	5.6±0.33	0.00±0.00	0.41±0.06	0.46±0.07	0.94±0.02	0.03774±0.0101	0.0020±0.0007
JC3C	0.009±0.003	2.02±0.16	3.66±0.20	5.7±0.27	0.01±0.01	0.03±0.02	0.35±0.14	0.36±0.02	0.0029±0.0011	0.0019±0.0007
JC31	0.014±0.006	1.91±0.15	4.60±0.27	6.5±0.38	0.01±0.01	0.01±0.01	0.52±0.26	0.30±0.03	0.0031±0.0013	0.0022±0.0010
JC34	0.006±0.004	1.69±0.12	0.12±0.05	1.7±0.19	0.01±0.00	0.07±0.02	0.18±0.14	0.92±0.04	0.0174±0.0066	0.0058±0.0043

*RP = $N_2O+N_2/N_2O+N_2+NO_3^-$-N

Appendix 11.b Mean dissolved N species and dissolved CO_2 and CH_4 gases in groundwater at SH during 2009-2010

Well ID	N_2O-N (mg N L^{-1})	Excess N_2-N (mg N L^{-1})	NO_3^--N (mg N L^{-1})	Total N Loadings (mg N L^{-1})	NO_2^- (mg N L^{-1})	NH_4^+ (mg N L^{-1})	DON (mg N L^{-1})	RP*	N_2O-N/NO_3^--N	N_2O/total N loadings
Subsoil										
SH1A	0.017±0.006	1.6±0.14	1.4±0.12	3.1±0.18	0.00±0.00	0.02±0.01	0.50±0.14	0.53±0.04	0.0240±0.0172	0.0058±0.0022
SH2A	0.001±0.000	2.6±0.29	0.04±0.01	2.5±0.46	0.00±0.00	0.03±0.01	0.04±0.02	0.93±0.05	0.0317±0.0238	0.0003±0.0001
SH3A	0.005±0.005	2.9±0.26	0.04±0.01	2.7±0.26	0.00±0.00	0.02±0.01	0.04±0.01	0.98±0.00	0.1438±0.0102	0.0015±0.0013
Interface										
SH1B	0.018±0.009	1.2±0.13	1.62±0.21	2.8±0.10	0.00±0.00	0.01±0.01	0.35±0.13	0.43±0.05	0.0158±0.0113	0.0059±0.0029
SH2B	0.005±0.002	4.4±0.22	0.07±0.05	4.5±0.18	0.00±0.00	0.02±0.01	0.12±0.14	0.99±0.01	0.2418±0.0125	0.0012±0.0006
SH3B	0.005±0.003	2.2±0.17	0.04±0.02	2.3±0.17	0.00±0.00	0.01±0.01	0.06±0.04	0.93±0.05	0.2184±0.0145	0.0026±0.0015
Bedrock										
SH1C	0.024±0.007	0.9±0.15	1.9±0.17	2.8±0.25	0.00±0.00	0.01±0.01	0.28±0.09	0.31±0.04	0.0118±0.0035	0.0083±0.0024
SH2C	0.003±0.002	3.3±0.24	0.11±0.04	3.4±0.17	0.00±0.00	0.03±0.01	0.02±0.01	0.97±0.01	0.0941±0.0067	0.0025±0.0025
SH3C	0.011±0.008	2.4±0.32	0.09±0.05	2.6±0.39	0.00±0.00	0.01±0.00	0.42±0.34	0.95±0.03	0.2336±0.0109	0.0071±0.0062

*RP = $N_2O+N_2/N_2O+N_2+NO_3^-$-N

Appendix 11.c Mean dissolved N species and dissolved CO_2 and CH_4 gases in groundwater at OP during 2009-2010

Well ID	N_2O-N (mg N L^{-1})	Excess N_2-N (mg N L^{-1})	NO_3^--N (mg N L^{-1})	Total N Loadings (mg N L^{-1})	NO_2^- (mg N L^{-1})	NH_4^+ (mg N L^{-1})	DON (mg N L^{-1})	RP*	N_2O-N/NO_3^--N	N_2O/total N loadings
Subsoil										
OP1A	0.031±0.005	0.3±0.1	9.0±0.7	9.3±0.9	0.00±0.00	0.01±0.01	0.51±0.24	0.07±0.05	0.0057±0.0030	0.0036±0.0006
OP2A	0.063±0.020	1.5±0.3	11.6±1.1	13.1±1.1	0.57±0.06	0.23±0.09	1.75±0.36	0.12±0.02	0.0056±0.0026	0.0067±0.0028
Interface										
OP1B	0.027±0.007	0.7±0.1	9.6±0.3	10.3±0.3	0.00±0.00	0.02±0.01	0.62±0.26	0.07±0.01	0.0028±0.0007	0.0026±0.0007
OP2B	0.042±0.009	0.8±0.1	12.5±0.6	13.3±0.6	0.77±0.35	0.03±0.02	2.15±0.49	0.06±0.01	0.0034±0.0006	0.0032±0.0005
Bedrock										
OP1C	0.042±0.009	1.1±0.2	11.4±0.3	12.6±0.5	0.01±0.01	0.02±0.01	1.07±0.29	0.09±0.01	0.0037±0.0008	0.0034±0.0008
OP2C	0.032±0.010	1.3±0.1	12.5±0.2	13.8±0.2	0.02±0.01	0.02±0.02	1.41±0.43	0.09±0.01	0.0026±0.0008	0.0023±0.0007

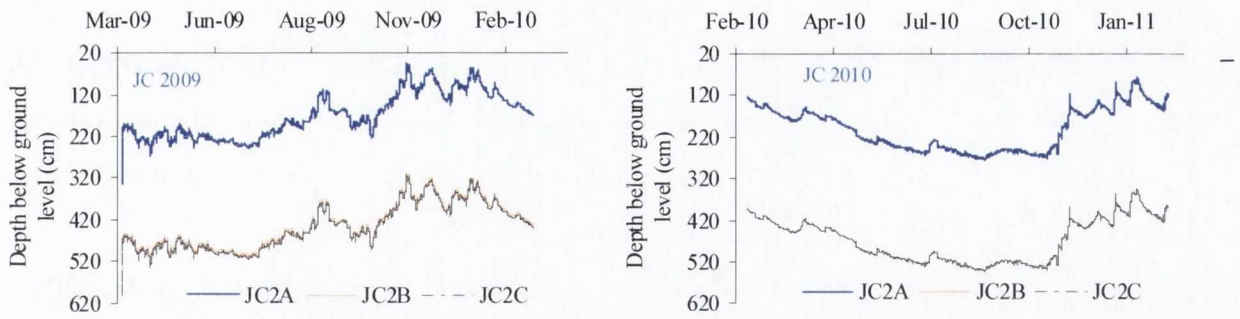
*RP = $N_2O+N_2/N_2O+N_2+NO_3^-$-N

Appendix 11.d Mean dissolved N species and dissolved CO_2 and CH_4 gases in groundwater at DG during 2009-2010

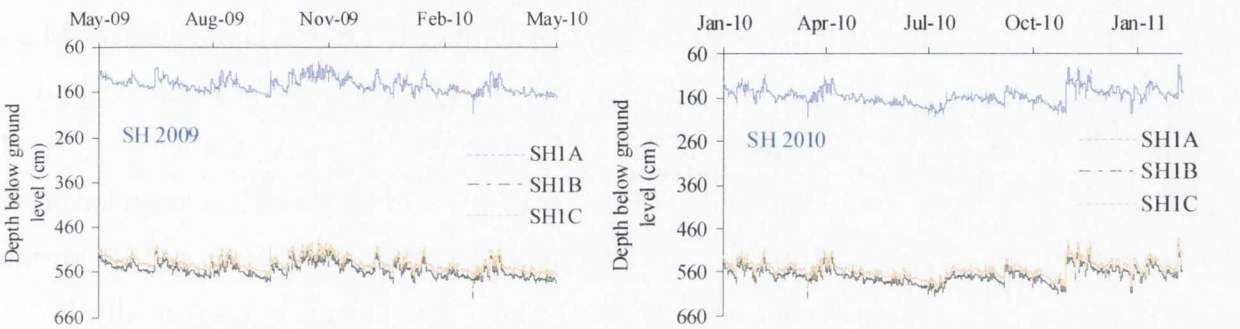
Well ID	N_2O-N (mg N L^{-1})	Excess N_2-N (mg N L^{-1})	NO_3^--N (mg N L^{-1})	Total N Loadings (mg N L^{-1})	NO_2^- (mg N L^{-1})	NH_4^+ (mg N L^{-1})	DON (mg N L^{-1})	RP*	N_2O-N/NO_3^--N	N_2O/total N loadings
DG101	0.039±0.007	0.5±0.1	17.8±1.1	18.3±1.3	0.00±1.1	0.02±0.00	1.34±0.01	0.03±0.45	0.0022±0.01	0.0022±0.0004
DG102	0.030±0.006	0.2±0.1	7.6±0.3	7.9±0.1	0.00±0.3	0.01±0.00	0.29±0.01	0.04±0.13	0.0039±0.01	0.0037±0.0007
DG103	0.033±0.006	0.5±0.1	9.0±0.8	9.5±0.8	0.00±0.8	0.01±0.00	0.92±0.01	0.06±0.59	0.0038±0.02	0.0036±0.0007
DG104	0.086±0.031	0.5±0.1	17.7±0.9	18.2±0.9	0.00±0.9	0.01±0.00	2.50±0.01	0.03±0.76	0.0050±0.01	0.0048±0.0016
DG105	0.039±0.005	0.6±0.1	12.6±0.5	13.3±0.4	0.00±0.5	0.01±0.00	1.99±0.00	0.05±0.74	0.0031±0.01	0.0030±0.0004
DG106	0.063±0.011	0.5±0.1	22.7±1.3	23.2±0.8	0.01±1.3	0.02±0.00	3.42±0.02	0.02±0.84	0.0030±0.00	0.0029±0.0005

*RP = $N_2O+N_2/N_2O+N_2+NO_3^-$-N

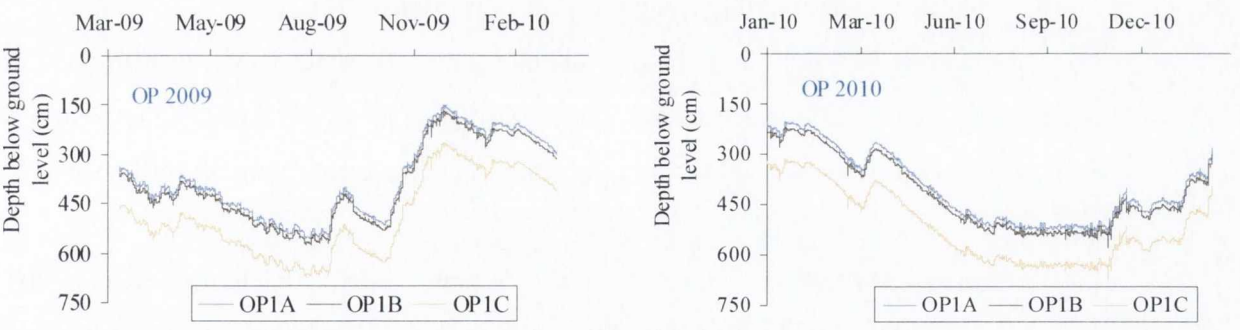
Appendix 12.a Groundwater table fluctuations in every 30 min at JC site



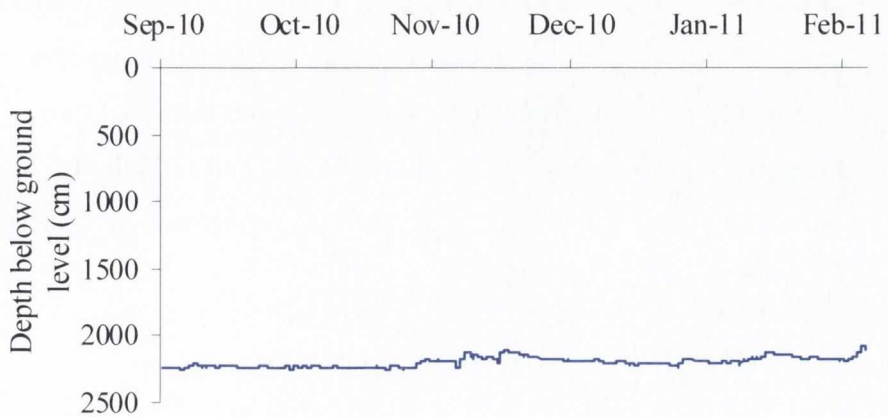
Appendix 12.b Groundwater table fluctuations in every 30 min at SH site



Appendix 12.c Groundwater table fluctuations in every 30 min at OP site



Appendix 12.d Groundwater table fluctuations in every 30 min at DG site



Appendix 13 Papers (published/to be published) in journals on this project

Jahangir, M.M.R., Khalil, M.I., Johnston, P.M., Cardenas, L.M., Butler, M., Hatch, D., Barrett, M., O'Flaherty, V., Richards, K.G. 2011. Denitrification potential in subsoils: a mechanism to reduce nitrate leaching to groundwater. Agriculture, Ecosystems and Environment, 147, 13-23.

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Richards, K.G. 2010. Assessing groundwater denitrification under two contrasting land uses in South East Ireland. Advances in Animal Biosciences, 1(1), 87, (DOI: 10.1017/S204047001000230X)

Jahangir, M.M.R., Johnston, P., Groffman, P., Khalil, M.I., Richards, K.G. 2011. In Situ Denitrification Capacity in Shallow and Deeper Groundwaters Measured Using Push-Pull Method. Journal of Environmental Quality (under revision).

Barrett, M., **Jahangir, M.M.R.**, Khalil, M.I., Lee, C., Cardenas, L.M., Collins, G., Richards, K., and O'Flaherty, V. 2011. Effect of carbon amendment and soil depth on the distribution of denitrifers and the emission of N_2 and N_2O using nirK, nirS, and nosZ functional markers. FEMS Microbial Ecology (submitted).

Jahangir, M.M.R., Johnston, P., Grant, J., Somers, C., Khalil, M.I., Richards, K.G. 2011. Evaluation of headspace equilibration methods for groundwater GHGs. Journal of Environmental Management (resubmitted).

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Barrett, M.M., Groffman, P.M. Richards, K.G. 2011. Denitrification, $N_2O/(N_2O+N_2)$ ratios and N_2O emission factors along groundwater flow paths: hydrologic and biogeochemical influences. Water Resour. Res. (submitted).

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Richards, K.G. 2011. Linking hydrogeochemistry to the abundances of nitrate in groundwater. Journal of Hydrology (resubmitted after minor revision).

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Richards, K.G. 2011. Groundwater: A pathway of terrestrial C and N losses and indirect GHGs emissions. Agriculture, Ecosystems and Environment (resubmitted after minor revision).

Barrett, M. **Jahangir, M.M.R.**, M.I. Khalil, Richards, K., and O'Flaherty, V. Connecting microbial denitrifying abundance and N_2/N_2O emissions in Irish Groundwater systems. Microbial Ecology (submitted).

Papers presented in conferences/workshops of international societies

Jahangir, M.M.R., Johnston, P., Richards, K.G. 2012. Terrestrial carbon and nitrogen losses and indirect greenhouse gas emissions via groundwater, 40th IAH Congress, Niagara Falls, Toronto, Canada (submitted).

Jahangir, M.M.R., Johnston, P., Richards, K.G. Terrestrial carbon and nitrogen losses and indirect greenhouse gas emissions via groundwater. Proceedings of the 17th N Workshop, Wexford, Ireland.

Jahangir, M.M.R., Minet, E., Johnston, P., Catherine, C., Richards, K.G. 2012. Shallow groundwater denitrification in a sandy aquifer beneath spring barley - cover crop rotation: insights from a ^{15}N tracer experiment. Proceedings of the 17th N Workshop, Wexford, Ireland.

Jahangir, M.M.R., Johnston, P., Groffman, P.M., Khalil, M.I., Richards, K.G. 2011. Simultaneous occurrences of denitrification and DNRA in groundwaters: An in situ ^{15}N tracer study. Abstracts, B51F-0459, AGU, San Francisco, California, USA.

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Richards, K.G. 2011. Quantifying in situ denitrification rates and N_2O/N_2O+N_2 ratios in groundwater using ^{15}N tracer technique. In: Proceedings of the international conference 'Catchment Science 2011', Dublin, Ireland, p.94.

Jahangir, M.M.R., Johnston, P., Khalil, M.I, Richards, K.G. 2011. Catchment-scale nitrate attenuation and indirect N_2O emissions along groundwater flow paths. In: Proceedings of the international conference 'Catchment Science 2011', Dublin, Ireland, p.95.

Jahangir, M.M.R., Johnston, P., Richards, K.G. 2011. Nitrate attenuation via denitrification in subsoils and groundwater. Paper presented in a workshop organised by the EPA Hydrometric Research Division, and UCD Urban Institute Ireland, 16 May 2011, Dublin, Ireland

Jahangir, M.M.R., Johnston, P., Richards, K.G. 2011. Denitrification in subsoils and groundwater in Ireland. Paper presented in the Technical Discussion Meeting (TDM) of IAH- Irish Chapter, GSI, 31 March 2011, Dublin

Jahangir, M.M.R., Barrett, M., Johnston, P., O'Flaherty, V., Khalil, M.I., Richards, K.G. 2010. Groundwater denitrification and denitrifier functional gene abundances at a range of hydrogeological settings in Ireland. Abstracts, B51B-0356, AGU, San Francisco, California, USA.

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Fenton, O., Richards, K.G. 2010. Groundwater denitrification across vertical hydrogeochemical gradients in an

agricultural catchment. In: Reactive N management for sustainable development-science, technology and policy. Book of Abstracts, 5th International Nitrogen Conference, New Delhi, India, p. 369.

Jahangir, M.M.R., Johnston, P., Khalil, M. I., Richards, K.G. 2010. Indirect emission of N_2O from Irish groundwater. In: 'A Climate for Change: Opportunities for Carbon-Efficient Farming', Book of Abstracts, Teagasc International Conference 2010, Dublin, Ireland, p. 65.

Jahangir, M.M.R., Khalil, M.I., Richards, K.G., Johnston, P., Cardenas, L., and Hatch, D. 2010. Denitrification and the $N_2/(N_20+N_2)$ ratios at various soil depths under grazed grassland in Ireland. Proceedings, Joint SAC and SEPA Biennial Conference, Edinburgh, Scotland, UK, pp. 282-287.

Jahangir, M.M.R., Khalil, M.I., Cardenas, L., Hatch, D., Johnston, P., Richards, K.G. 2010. Can subsoil denitrification reduce groundwater nitrate pollution and atmospheric N_2O emissions? Geophysical Research Abstracts, Vol. 12, EGU2010-853-3.

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Richards, K.G. 2010. Shallow groundwater denitrification capacity at three contrasting hydrogeological environments in Ireland. Geophysical Research Abstracts, Vol. 12, EGU2010-857-5.

Jahangir, M.M.R., Johnston P.M., M.I. Khalil, Richards, K.G. 2010. Comparison of two sampling methods for groundwater dissolved gas analysis. Proceedings, 20th Annual Irish Environmental Researcher's Colloquium, LIT, Ireland, pp. 88-89.

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Richards, K. 2009. Extent and potential of groundwater denitrification in different hydrogeological and geochemical situations of Ireland. In: Proceedings of the 19th Annual Irish Environmental Researcher's Colloquium, WIT, Ireland, p. 97.

Jahangir, M.M.R., Johnston, P., Khalil, M.I., Richards, K.G. 2010. Assessing groundwater denitrification under two contrasting land uses in South East Ireland. In: proceedings of the international conference of British Animal Science Society and Agricultural Research Forum held in April 2010, Queens University Belfast, p.87.

Training courses participated during the study period

1. Training on "A Beginner's Guide to EndNote X3 for PC" on 26th March 2010 at Trinity College Dublin, Ireland.

2. A short course on "Stable isotope hydrology-with special focus on hydrogen and oxygen stable isotopes" organized by the European Geosciences Union subdivision on 'Catchment Hydrology' Technical University Vienna on May 2, 2010, Vienna, Austria.
3. A short course on 'Why (and how) to write a scientific paper in hydrology' and 'Meet the expert in hydrology' organized by the European Geosciences Union at the Austria Centre Vienna on May 5, 2010, Vienna, Austria.
4. Training on Health and Safety Assessment (GLP) for laboratory work, 16 September, 2009, Teagasc Environment Research Centre, Johnstown Castle, Ireland.
5. Training on Statistics for Research Students, 11-15 May and 22-26 June, 2009, Department of Statistics, School of Engineering, Mathematics and Science, Trinity College Dublin, Ireland. Training on statistics for research students in light with the use of SAS (SAS Inc, USA) during 8, 14 and 16 July, 2009, Teagasc Environment Research Centre, Johnstown Castle, Ireland.
6. Methodology training and measurement of biogenic gas emissions from soil using automated laboratory incubation technique, 27 March-13 July, 2008, Rothamsted Research, North Wyke, UK.
7. Training on Cost Assessment for Laboratory Equipment and General Lab Precautions, 29th March, 2008, Rothamsted Research, North Wyke, UK.
8. A short course on the Practical Guide to Hydrogeology, 3 September, 2008, White Yellow Green (WYG), Dublin, Ireland.
9. Training on Introduction to Gas Chromatography during 15-16 November, 2008, Teagasc Environment Research Centre, Johnstown Castle, Ireland.