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Denitrification and indirect N₂O emissions in groundwater: hydrologic and biogeochemical influences

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ABSTRACT

Identification of specific landscape areas with high and low groundwater denitrification potential is critical for improved management of agricultural nitrogen (N) export to ground and surface waters and indirect nitrous oxide (N₂O) emissions. Denitrification products together with concurrent hydrogeochemical properties were analysed over two years at three depths at two low (L) and two high (H) permeability agricultural sites in Ireland. Mean N₂O-N at H sites were significantly higher than L sites, and decreased with depth. Conversely, excess N₂-N were significantly higher at L sites than H sites and did not vary with depth. Denitrification was a significant pathway of nitrate (NO₃⁻-N) reduction at L sites but not at H sites, reducing 46-77% and 4-8% of delivered N with resulting mean NO₃⁻-N concentrations of 1-4 and 12-15 mg N L⁻¹ at L and H sites, respectively. Mean N₂O-N emission factors (EF_{5g}) were higher than the most recent Intergovernmental Panel on Climate Change (IPCC, 2006) default value and more similar to the older IPCC (1997) values. Recharge during winter increased N₂O but decreased excess dinitrogen (excess N₂-N) at both sites, probably due to increased dissolved oxygen (DO) coupled with low groundwater temperatures. Denitrifier functional genes were similar at all sites and depths. Data showed that highly favourable conditions prevailed for denitrification to occur - multiple electron donors, low redox potential (Eh <100 mV), low DO (<2 mg L⁻¹), low permeability (k_s<0.005 m d⁻¹) and a shallow unsaturated zone (<2 m). Quantification of excess N₂-N in groundwater helps to close N balances at the local, regional and global scales.

Keywords: denitrification, excess N₂-N, groundwater, emission factor, permeability, redox-potential

1. Introduction

Groundwater nitrate (NO_3^- -N) contamination is of global environmental concern and a health concern mainly due to its potential connection with eutrophication, hypoxia in estuaries (Rabalais, 2002) and methaemoglobinaemia in humans (WHO, 2004). Mitigation of NO_3^- -N contamination in groundwater generally relies on natural attenuation processes, which requires a comprehensive, quantitative understanding of denitrification, the main NO_3^- -N depletion mechanism in groundwater (Boyer et al., 2006; Rivett et al., 2008). Denitrification is a multistep biological process producing nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) from NO_3^- -N and is carried out mainly by facultative anaerobes.

Internationally there is great interest in the intermediate product of denitrification N_2O (Von der Heide et al., 2008), due to its contribution to stratospheric ozone depletion and radiative forcing (Townsend et al., 2003). There are large uncertainties with respect to the global budget of N_2O , and it is unclear which drivers are responsible for observed increases in atmospheric N_2O concentrations (Ehhalt et al., 2001). The production, movement and consumption of N_2O in groundwater are particularly poorly understood (Clough et al., 2007) and require further research with respect to the controls of N_2O production and reduction in groundwater (Well and Butterbach-Bahl, 2010). The Intergovernmental Program on Climate Change (IPCC) suggests that 0.25% (range 0.05 to 2.5%) of fertilizer N inputs to cropped fields can be emitted as N_2O from groundwater. There is great uncertainty surrounding this indirect emission factor ($\text{EF}_{5\text{g}}$) and it needs to be verified over a range of hydrogeological environments (IPCC, 2006). The amount of reactive N (N_r) that is converted back to N_2 is by far the largest uncertainty in the N cycle at all scales (Galloway et al., 2004), a fact that

restricts our ability to both manage and predict the consequences of an increasingly N-rich world (Townsend and Davidson, 2006).

Denitrification of NO_3^- -N migrating from cropped fields to groundwater along diverse hydrologic flow paths is particularly challenging to assess and model. The efficiency of NO_3^- -N removal by denitrification in groundwater ranges from 0% to 100% and depends on aquifer hydrology and mineralogy (Hiscock et al., 2003), hydrologic flow paths, dissolved oxygen (DO), microbial community composition, energy sources and redox chemistry (Boyer et al., 2006). A particular challenge to the construction and validation of robust and predictive models of denitrification arises from the fact that controlling factors are highly variable in space and time, giving rise to “hot spots” and “hot moments” of activity that are difficult to predict (McClain et al., 2003). Nitrate is one of the common contaminants of groundwater in Ireland but significant variation in hydrogeological conditions and agricultural practices can create marked variation in NO_3^- -N concentrations across the country. To implement the European Union Groundwater Directive, Nitrate Directive and Kyoto protocol, there must first be a basic scientific understanding of how hydrobiogeochemical conditions influence NO_3^- -N fate and transformation in different settings. In the current study, our objectives were (a) to quantify the extent of denitrification by measuring N_2O -N and excess N_2 -N in groundwater along three diverse, vertical hydrogeochemical gradients at four sites; and (b) to link N_2O -N and excess N_2 -N to hydrologic and biogeochemical parameters at the sites.

2. Materials and methods

2.1 Study sites

The investigation was carried out within four agricultural sites that represent a range of hydrobiogeochemical conditions and have contrasting groundwater NO_3^- -N and DO levels.

Two sites had low permeability, low DO and low NO_3^- -N termed as L; (L1) Johnstown Castle and (L2) Solohead. Two sites had higher permeability, high DO and high NO_3^- -N termed as H; (H1) Oak Park and (H2) Dairy Gold. The farm areas of L1, L2, H1 and H2 were 48, 32, 10 and 93 ha, respectively. An overview of soil type, bedrock geology, land uses and other geochemical parameters for these sites are summarised in Table 1. The soil drainage class of the L1 and L2 sites represents approximately 37% of the land area of Ireland (moderate to poor drainage), whereas well drained sites H1 and H2 cover approximately 62% of Ireland (Conry, 2006). Bedrock at the L1 and L2 sites is poorly productive and represents 71% of the area of Ireland, whereas at H1 and H2, the aquifer is sand and gravel and Karstified limestone that when combined covers 21.6% of the country (Daly, 2005).

2.2 Monitoring well establishment

Thirty specifically designed multilevel piezometers (5 cm Inner Diameter, ID with a 2 or 3 m screen section) were installed at three depths: 5, 10 and 20 m below ground level (bgl) representing the subsoil, bedrock-interface and bedrock, respectively at L1, L2 and H1. A groundwater monitoring network at each site was developed with 15, 9 and 6 wells at L1, L2 and H1, respectively to sample along flow paths. At H2, 6 single wells were established in the bedrock (30-50 m bgl; 6 m screen section) as no shallower water tables were encountered during drilling. Well locations across each site were at least 200 m apart and were chosen to minimize hydrogeological heterogeneity and to ensure the availability of groundwater. Well integrity was checked for each borehole by adding 5 L water to increase the static water level to 1 m height in one well whilst measuring water level changes in the two adjacent wells using an electronic transducer (Diver, Eijkelkamp, The Netherlands).

2.3 Groundwater sampling

Groundwater sampling was carried out monthly over two years (February, 2009 to January, 2011) using a bladder pump (Geotech Environmental Equipment, Inc., USA) following USEPA Region I Low Stress Sampling Procedures (USEPA, 1996). Triplicate samples were collected through a Teflon water outlet tube (ID 0.6 cm) at a slow rate (100 ml min⁻¹) to avoid ebullition of dissolved gases during sampling. To analyse dissolved N₂-N, Argon (Ar) and DO, samples were collected into a 12 ml exetainer (Labco Ltd, Wycombe, UK) by slowly overflowing approximately 10 ml excess water and then immediately sealing using double septum (butyl rubber + Teflon) stoppers. To analyse dissolved N₂O-N, water samples were collected into 160 ml serum bottles by overflowing approximately 150 ml water and then immediately sealing with butyl rubber septa and aluminium crimp caps (WHEATON, USA). All samples, including samples for dissolved gases (stored under water), were stored at 4°C and analysed within one week.

2.4 Hydrology

The thickness of the unsaturated zone was quantified by measuring changes in groundwater table (GWT) depth below the ground surface. Monthly GWT changes were measured manually by an electronic dip meter prior to the sampling of groundwater. Saturated hydraulic conductivity (k_s – m d⁻¹) for screened intervals was estimated using the slug test procedures described by Bouwer and Rice (1976).

2.5 Hydrogeochemistry

Groundwater pH, temperature, DO, electrical conductivity (EC) and redox potential (Eh) were measured using an In Situ Multiparameter Probe (In Situ Inc. Ltd., USA). Non-

metallic ions e. g. total oxidised N, ammonium (NH_4^+), chloride (Cl^-), and orthophosphate (PO_4^{3-}); reduced metals (Fe^{2+} , Mn^{2+}) and S^{2-} (sulphide) were analyzed with an Aquakem 600 Discrete Analyser (Aquakem 600A, Vantaa, Finland). Sulphate (SO_4^{2-}) concentration was measured with a turbimetric method (Askew and Smith, 2005a). Dissolved Organic Carbon (DOC) was analysed using a Total Organic Carbon Analyser (TOC-V cph/cpn; Shimadzu Corporation, Kyoto, Japan) and total N and total PO_4^{3-} were analysed using a persulfate method (Askew and Smith, 2005b).

2.6 Measurement of dissolved gases in groundwater

The exetainer samples for $\text{N}_2\text{-N}$, DO and Ar were analysed in a high precision membrane inlet mass spectrometer (MIMS) (Kana et al., 1994). To determine the dissolved $\text{N}_2\text{O-N}$, samples were degassed using high purity He (BOC, Linde Group, Germany) (He: water 1:3; v/v) following the headspace equilibration technique. $\text{N}_2\text{O-N}$ was analysed by electron capture gas chromatography (CP-3800, Varian, Inc. USA) using Ar as a carrier gas.

2.7 Estimating dissolved $\text{N}_2\text{O-N}$ and excess $\text{N}_2\text{-N}$

The $\text{N}_2\text{O-N}$ concentrations in water samples were estimated using Henry's Law. The partial pressures of $\text{N}_2\text{O-N}$ 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 the groundwater surface. Denitrified $\text{N}_2\text{-N}$, presented as excess $\text{N}_2\text{-N}$ (Heaton and Vogel, 1981), was estimated following the method described by Weymann et al. (2008).

2.8 Estimation of initial N concentration, N_2O -N EF_{5g} , N_2O -N mole fraction and reaction progress (RP)

Using the assumption that any changes to NO_3^- -N concentrations along a groundwater flow path between the aquifer surface and a given sampling spot location were due to denitrification and that this results in the quantitative accumulation of gaseous denitrification products, it follows that the initial N concentration can be calculated from the sum of residual substrates and the accumulated products (Böhlke, 2002).

The initial N concentration can be estimated using Equation 1:

$$\text{Total } N_{ini} = N_2O-N + \text{Excess } N_2-N + NO_3^- - N + NO_2^- - N + NH_4^+ + \text{DON} \quad (1)$$

where DON is dissolved organic N calculated by subtracting inorganic N from total N. Reaction progress (RP) was estimated as the ratio between the products and starting substrates (Weymann et al., 2008) and was used to characterise the extent of NO_3^- -N elimination by denitrification using Equation 2:

$$RP = \frac{TDN}{\text{Total } N_{ini}} \quad (2)$$

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

The N_2O -N EF_{5g} for indirect N_2O -N emissions from groundwater was estimated using the method described by Weymann et al., (2008) and calculated using Equation 3:

$$EF_{5g}(1) = \frac{N_2O-N}{\text{Total } N_{ini}} \quad (3)$$

An alternative EF_{5g} , as proposed by many researchers (Reay et al., 2003; Sawamoto et al., 2003) and adopted by the IPCC (2006), assumes that NO_3^- -N and N_2O -N are not transformed during transport into and through aquifers was calculated using Equation 4:

$$EF_{5g}(2) = \frac{N_2O-N}{NO_3^- - N} \quad (4)$$

The N₂O-N mole fraction was estimated using Equation 5:

$$\text{Mole fraction} = \text{N}_2\text{O-N}/\text{TDN} \quad (5)$$

2.9 Farm scale N balance approach

A water balance was used to calculate a farm scale N balance. The volume of effective rainfall (L) that recharged groundwater over a hydrological year was assumed equal to the volume of groundwater that was discharged to surface waters, because groundwater storage is assumed to be zero (Fitzsimons and Misstear, 2006). To estimate effective rainfall, the modified Penman-Monteith equation (Allen et al., 1998) and the hybrid soil moisture deficit model of Schulte et al. (2005) for Irish grasslands were used to process the potential evapotranspiration and actual evapotranspiration, respectively. The amount of dissolved N leached from soil to groundwater was assumed to be equal to Total N_{ini} drained from groundwater to surface water, because mean Total N_{ini} in groundwater in two distinct hydrological years was basically consistent. The quantity of dissolved N₂O-N or Total N_{ini} (QTotal N_{ini}) drained from groundwater to surface water was estimated using Equation 6:

$$Q\text{Total } N_{ini} = Q_{ER} * \bar{\chi} \text{Total } N_{ini} \text{ conc.} * 10^{-6} \quad (6)$$

where Q_{ER} is the volume of groundwater discharge per year and the concentration of N was multiplied by 10⁻⁶ to convert mg to kg. Indirect N₂O-N emissions (%) were estimated using Equation 7:

$$\text{Indirect } N_2O \text{ emissions} = \frac{N_2O \text{ lost from groundwater to surface waters}}{\text{Total } N \text{ input}} * 100 \quad (7)$$

where N₂O-N lost from groundwater to surface waters was in kg N ha⁻¹y⁻¹, and total N input included fertilizer N, organic N (slurry and dairy soiled water) and imported concentrated

animal feed ($\text{kg N ha}^{-1}\text{y}^{-1}$). The $\text{N}_2\text{O-N}$ $\text{EF}_{5\text{g}}$ for groundwater was estimated using Equation 8:

$$\text{EF}_{5\text{g}} = \frac{\text{N}_2\text{O lost from groundwater to surface waters}}{\text{Total N}_{\text{ini}} \text{ lost from groundwater to surface waters}} \quad (8)$$

2.10 Denitrifier functional genes in groundwater

Groundwater samples of 5 L were collected from each well in May and June 2009 for analysis of the abundance of denitrifier functional genes (*nirK*, *nirS* and *nosZ*) at the National University of Ireland in Galway (Barrett et al., 2010). The DNA was concentrated by vacuum filtration on 0.2 μm filter paper. Functional gene abundance was quantified using real-time PCR assays targeting the NO_2^- reductase (*nir*) and N_2O reductase (*nos*) genes.

2.11 Statistical analysis

Data were analyzed using the Mixed Procedure in the Statistical Analysis System (SAS, 2009). As most of the variables were lognormally distributed, log transformations were used with appropriate re-scaling. Pre-specified hypotheses of influential variables were tested by regression modelling for both N_2O and excess N_2 . Covariance models were included to account for correlations in the data (e.g. across sampling dates). For the concentration of denitrification end products, N_2O mole fractions and emission factors, the effects of location and depth were examined along with their interactions. Where significant differences between factors were found, the Tukey Kramer HSD all pairs multiple comparison test was used to distinguish specific differences.

3. Results

3.1 Site and temporal variability in $\text{N}_2\text{O-N}$ and excess $\text{N}_2\text{-N}$ concentrations

Irrespective of depth, mean $\text{N}_2\text{O-N}$ concentrations at H sites were significantly higher ($p < 0.05$) than at L sites, with no significant differences within site class ($p > 0.05$). Mean N_2O concentrations were 0.024, 0.011, 0.038 and 0.049 mg N L^{-1} at the L1, L2, H1 and H2 sites respectively. Within each site, N_2O concentrations were similar among multilevel depths ($p > 0.05$) except at L1 where they were significantly higher in the subsoil than at the interface and bedrock zones (Fig 1). Temporal variation in $\text{N}_2\text{O-N}$ concentrations was moderate across sites and depths with significantly higher ($p < 0.05$) concentrations in February and March than other sampling dates. A remarkably high site variability in $\text{N}_2\text{O-N}$ concentrations was observed at all sites and depths. Coefficients of variation between depths ranged from 87-126, 91-149, 56-81 and 50-82% at L1, L2, H1 and H2, respectively.

Mean excess $\text{N}_2\text{-N}$ concentrations in groundwater were 2.28, 2.33, 0.90 and 0.46 mg N L^{-1} at L1, L2, H1 and H2 sites, respectively (Fig 2). Irrespective of depth, mean excess $\text{N}_2\text{-N}$ concentrations were significantly higher at L ($p < 0.05$) than at H sites with no significant differences within site class ($p > 0.05$). Excess $\text{N}_2\text{-N}$ concentrations were similar among depths except at H1 where they were significantly higher in the bedrock than in the subsoil and interface zones ($p < 0.001$). Excess $\text{N}_2\text{-N}$ concentrations were moderately variable over time with highest concentrations observed in July to October and lowest in November to February regardless of sites and depth. In November and December they were significantly lower ($p < 0.05$) than at other sampling times. Excess $\text{N}_2\text{-N}$ had large spatial variability, with coefficients of variation between depths ranging from 55-82, 45-65, 36-69 and 74% at L1, L2, H1 and H2 sites, respectively.

3.2 Groundwater as a source of atmospheric $\text{N}_2\text{O-N}$: mole fractions and EF_{5g}

Mean $\text{N}_2\text{O-N}$ mole fractions were 0.02, 0.01, 0.08 and 0.19 at L1, L2, H1 and H2 sites, respectively (Table 2). Nitrous oxide mole fraction was significantly higher at H sites ($p < 0.001$) than at L sites. No significant difference was observed within the sites ($p > 0.05$). Nitrous oxide mole fraction was significantly higher in subsoil than at interface and bedrock zones at L1 and H1 ($p < 0.001$) sites but at the L2 site it was similar among depths. $\text{N}_2\text{O-N}$ mole fraction significantly decreased as excess $\text{N}_2\text{-N}$ increased (Fig 3) indicating that $\text{N}_2\text{O-N}$ was further reduced to N_2 in groundwater. Mean $\text{N}_2\text{O-N}$ $\text{EF}_{5\text{g}}(2)$ based on ambient $\text{NO}_3^- \text{-N}$ ranged from 0.003 at the H2 site to 0.029 at the L2 site (Table 2). Using the method proposed by Weymann et al. (2008), the $\text{N}_2\text{O-N}$ $\text{EF}_{5\text{g}}(1)$ ranged from 0.003 at L to 0.004 at H sites (Table 2). The $\text{EF}_{5\text{g}}$ according to the farm balance approach was similar to the $\text{EF}_{5\text{g}}(1)$. Indirect $\text{N}_2\text{O-N}$ emissions as a fraction of N input were lower at L sites than at H sites (Table 2). The $\text{EF}_{5\text{g}}$ differed significantly between sites ($p < 0.05$) but not among depths at each site ($p > 0.05$).

3.3 Initial N loadings and $\text{NO}_3^- \text{-N}$ removal by denitrification

Mean Total N_{ini} concentration (N delivered to groundwater from soil) varied significantly between sites ($p < 0.001$). The concentrations were similar among depths except at the H1 site where it was significantly higher ($p < 0.001$) in bedrock than in the subsoil or at the interface zone (Table 2). Sites L1 and L2 showed significantly lower N_{ini} than H1 and H2 but no significant differences were observed within site permeability class (L or H). Mean TDN differed significantly between sites ($p < 0.001$) except between L1 and L2 (Table 2). The TDN did not differ significantly between depths at each site ($p > 0.05$) except at H1 where it was significantly higher in bedrock than in the subsoil or interface zones ($p < 0.01$). Mean losses of initial N by denitrification, expressed by the RP was lower at H than at L sites

(Table 2). There was a significant difference between sites ($p<0.001$) but no significant differences with depth, except at the L1 site where RP was significantly lower in the subsoil than at the interface ($p<0.001$) or bedrock zones ($p<0.001$). There was a negative correlation between ambient NO_3^- -N and mean RP ($R^2=0.94$, $p<0.01$), TDN ($R^2=0.51$, $p<0.01$) and excess N_2 -N ($R^2=0.52$, $p<0.01$) (Fig. 4).

3.4 Abundances in denitrifier functional genes in groundwater

Denitrifier functional genes in groundwater were detected across all sites and depths with no significant differences ($p>0.05$) between sites and depths (Fig 5). The most abundant denitrifying functional gene was *nirS* (nitrite reductase), which ranged from 1.4×10^4 genes L^{-1} in the subsoil to 1.2×10^4 genes L^{-1} in the bedrock followed by *nosZ* (nitrous oxide reductase), which varied from 1.1×10^3 genes L^{-1} in the subsoil to 1.9×10^3 genes L^{-1} at the interface. The *nirK* (nitrite reductase that contains copper; Cu-Nir) functional gene was consistently lower at the interface (3.8×10^1 genes L^{-1}) than in the subsoil (6.2×10^1 genes L^{-1}) and in the bedrock zone (1.5×10^2 genes L^{-1}). The denitrifier genes to bacteria ratios $\{(nirK+nirS+nosZ)/\text{bacteria}\}$ were similar ($p>0.05$) across sites and among depths at each site, ranging from 0.60 ± 0.06 , 0.70 ± 0.22 , 0.39 ± 0.15 and 0.58 ± 0.25 , at L1, L2, H1 and H2 sites, respectively.

3.5 Environmental drivers of groundwater denitrification

Groundwater N_2O concentration increased with the depth of the GWT ($r=0.604$, $p=0.001$) and k_s values ($r=0.394$; $p=0.013$). There were significant positive linear relationships between N_2O -N and 1) Eh ($r=0.803$; $p=0.001$) 2) DO ($r=0.676$; $p=0.001$) 3) DOC ($r=0.374$; $p=0.011$), 4) NO_3^- -N ($r=0.809$; $p=0.001$) and 5) SO_4^{2-} ($r=0.416$; $p=0.006$).

Groundwater pH ($r=-0.405$; $p=0.007$), $\text{NH}_4^+\text{-N}$ (-0.238 ; $p=0.051$), excess $\text{N}_2\text{-N}$ ($r=-0.401$; $p=0.008$) and RP ($r=-0.769$; $p=0.001$) increased significantly with decreasing $\text{N}_2\text{O-N}$ concentrations. Overall *nir/(nir+nosZ)* and *nosZ* gene abundance were not significantly correlated with $\text{N}_2\text{O-N}$. Stepwise multiple linear regression produced a best fit model where log Eh, log $\text{NO}_3^-\text{-N}$, and log DOC showed positive relationships with predicted $\text{N}_2\text{O-N}$ concentrations (Table 3). In addition, when all the sampling dates were included into the model, February and March had significantly higher ($p<0.01$) $\text{N}_2\text{O-N}$ than other sampling dates.

Excess $\text{N}_2\text{-N}$ in groundwater was negatively correlated ($r=-0.474$; $p=0.003$) with the depth of the GWT and k_s ($r=-0.256$; $p=0.050$) and positively correlated with T °C ($r=0.405$; $p=0.004$). Excess $\text{N}_2\text{-N}$ decreased with increasing DO ($r=-0.667$; $p=0.001$) and Eh ($r=-0.631$; $p=0.001$). Groundwater $\text{NH}_4^+\text{-N}$ was positively correlated with excess $\text{N}_2\text{-N}$ ($r=0.301$; $p=0.048$). The DOC was negatively correlated ($r=-0.402$; $p=0.01$) and SO_4^{2-} concentration was positively correlated ($r=0.32$; $p=0.04$) with excess $\text{N}_2\text{-N}$. Conversely, excess $\text{N}_2\text{-N}$ showed significant positive correlation with Fe^{2+} ($r=0.66$; $p=0.001$) and Mn^{2+} ($r=0.75$; $p=0.001$). Nitrate-N, $\text{NO}_2^-\text{-N}$ and $\text{N}_2\text{O-N}$ were negatively correlated with excess $\text{N}_2\text{-N}$ with r values of -0.484 , -0.429 and -0.401 ($p=0.002$, 0.005 and 0.008 , respectively). Groundwater denitrifier abundances e.g. *nir/(nir+nosZ)* showed negative ($r=-0.232$; $p=0.057$) whereas *nosZ* showed positive relationships ($r=0.398$; $p=0.012$) with excess $\text{N}_2\text{-N}$. Stepwise multiple regression highlighted negative relationships between log-excess $\text{N}_2\text{-N}$ and logDO, log $\text{NO}_3^-\text{-N}$ and logDOC (Table 3). The sampling dates had a significant role on the predicted excess $\text{N}_2\text{-N}$ concentrations causing substantial changes to the intercept of the model (lower in November and December than other sampling dates).

4. Discussion

4.1 Indirect N_2O -N emissions

Results showed that N_2O -N in groundwater can originate from 1) recharge from surface soils or 2) can be produced *in-situ*. A ^{15}N tracer test carried out at the L1 and H1 sites showed that both N_2O -N and N_2 -N are produced *in-situ* in groundwater (Jahangir et al., 2013). Higher N_2O -N concentrations at the H1 and H2 sites were likely due to unfavourable conditions for further transformation of N_2O at those sites; a comparatively thick unsaturated zone, high permeability and low anaerobicity. These conditions directly inhibit reduction of N_2O -N and result in high NO_3^- -N concentrations. We observed N_2O -N production at a wide range of DO suggesting that N_2O -N production in comparatively aerobic conditions in groundwater might take place in microsites. Aerobic denitrification in soils (~80% air saturation) was reported by Carter et al. (1995), but in groundwater denitrification actually is more likely to occur 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). Denitrification may cease 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 -N can be a dominant product of denitrification.

Transformations of both NO_3^- -N to N_2O -N and N_2O -N to N_2 -N increased with depth and resulted in the low N_2O -N accumulation in the interface and bedrock zones (Fig. 1). Higher N_2O -N 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 -N production and accumulation at 2 to 3 m relative to 6.5 to 7.0 m below water table. Deurer et al. (2008) identified a zone of considerable N_2O -N accumulation close to the groundwater surface. Von

der Heide (2008) showed that the shallow groundwater zone was an active zone of heterotrophic denitrification. Weymann et al. (2009; 2010) and Von der Heide et al. (2009) concluded that $\text{N}_2\text{O-N}$ accumulation resulted from *in-situ* production in groundwater. Correlations between $\text{N}_2\text{O-N}$ and excess $\text{N}_2\text{-N}$ and ambient hydrogeochemical properties (discussed below in section 4.6) indicated that groundwater $\text{N}_2\text{O-N}$ was both produced and reduced to $\text{N}_2\text{-N}$ *in-situ* at our sites.

Annual variation in the hydrologic regime provided significant insight into the environmental controls on groundwater denitrification. Rainfall was lower in 2010 (mean $\pm$ SE: 864 $\pm$ 147 mm) compared with 2009 (mean $\pm$ SE: 1329 $\pm$ 229 mm) and as a result, groundwater recharge was lower in 2010 (mean $\pm$ SE: 320 $\pm$ 27 mm) than in 2009 (mean $\pm$ SE: 701 $\pm$ 59 mm). In the drier year (2010), $\text{N}_2\text{O-N}$ was lower than in the wetter year (2009), and the opposite was observed for $\text{N}_2\text{-N}$ (Fig 1 and 2). Von der Heide et al. (2009) argued that a drop in the GWT causes a decrease in $\text{N}_2\text{O-N}$ production whereas recharge stimulates $\text{N}_2\text{O-N}$ production. Recharge can increase groundwater DO, DOC and NO_3^- -N resulting in increased $\text{N}_2\text{O-N}$ production and could decrease further $\text{N}_2\text{O-N}$ reduction (Davidson, et al., 1993). Deurer et al. (2008) suggested that an increase in DO with recharge, coupled with low temperature decreased the potential for $\text{N}_2\text{O-N}$ reduction and increased its accumulation. The effect of DO on N_2O production is also illustrated by the higher N_2O concentrations at H sites (DO > 6.5 mg L⁻¹) than L sites (DO < 2.0 mg L⁻¹).

Groundwater $\text{N}_2\text{O-N}$ concentrations varied more in space than in time; $\text{N}_2\text{O-N}$ concentrations exhibited a high coefficient of variation (mean CV = 121, 180, 162 and 82%, respectively in L1, L2, H1 and H2). High spatial variability of groundwater N_2O concentrations were also reported by other researchers e.g. a CV value of 219% at the farm

scale (Von der Heide et al., 2008), 258% at the aquifer scale and 19 – 109% at the transect scale (Von der Heide et al., 2009).

4.2 Groundwater complete denitrification: Excess N_2 -N

Excess N_2 -N concentrations in our study sites were lower than those reported by Weymann et al. (2008). Maximum values in the present study were between 5.60 – 8.69 mg N L⁻¹ at the L1 and L2 sites and were associated with high RP values (between 0.97 – 0.99) at the interface and bedrock zones. Such values were slightly higher than those reported by Böhlke et al. (2007) who found a highest value of 5.88 mg N L⁻¹ excess N_2 -N in NO_3^- -N contaminated groundwater in Nebraska, USA. The high RP values that we observed were consistent with very low NO_3^- -N concentrations, indicating the occurrence of complete denitrification. The higher excess N_2 at L1 and L2 indicated that complete denitrification required high anaerobicity e.g. DO <2.0 mg L⁻¹, Eh $\pm$ 100 and an electron donor such as DOC or reduced Fe^{2+}/S^{2-} . Low DO and Eh at L sites are consistent with high DOC (Table 1). Conversely, lower excess N_2 -N was observed at sites H1 and H2, which had higher DO concentrations (6.0 - 9 mg L⁻¹) and Eh (150-250 mV), with DOC and reduced S^{2-} and Fe^{2+} acting as available electron donors. An unusually high pH (7 - 10) at the H1 site could also have contributed to low denitrification as Rust et al. (2000) suggested denitrification tends to be low above pH 8.3. Similar excess N_2 -N concentrations across all depths at the L1 and L2 sites indicated that denitrification can take place along groundwater flow paths from surface N sources to receiving areas at depth (Konrad, 2007) and is not confined to the upper layer. We observed higher denitrification potential in bedrock than in subsoil ($p < 0.05$) at the H1 site. These results are consistent with those of Weymann et al. (2008) who found lower excess N_2 -N in shallow groundwater compared to groundwater 5 m below the water table.

Weymann et al. (2008) differentiated process zones of heterotrophic and autotrophic denitrification in the shallow groundwater zone and in the zone beyond 5 m below the water table, respectively. Kolle et al. (1985) and Weymann et al. (2010) postulated that high NO_3^- -N removal in autotrophic denitrification zones was most likely caused by practically anoxic conditions and the presence of highly reactive microcrystalline pyrite. In the present study, the temporal variation of excess N_2 -N was low relative to site variability, with higher concentrations from July to October than at other times of the year. A similar pattern of NO_3^- -N removal in riparian groundwater was observed by Maître et al. (2003) and Curie et al. (2009) who found highest NO_3^- -N removal in spring and lowest in winter. As in our study and others (e.g. Baily et al., 2011), spatial variability was more pronounced than temporal variability, highlighting that groundwater is a heterogeneous system.

4.3 Groundwater as a source of atmospheric N_2O -N: mole fractions and EF_{5g}

The N_2O -N mole fraction ($\text{N}_2\text{O-N}/(\text{N}_2\text{O-N} + \text{excess } \text{N}_2\text{-N})$) in the subsoil at the H1 site and in the bedrock at the H2 site was very high (0.08 and 0.19), which was not surprising given that excess N_2 -N concentrations were very low at these locations due to unfavourable hydrochemical conditions for complete denitrification (discussed in 4.4). These results are comparable with the mole fractions reported by Weymann et al. (2010) (0.004 to 0.41). High N_2O -N mole fractions can occur due to low N_2O -N reduction at high NO_3^- -N concentrations. Magalhaes et al. (2003) showed an increase in $\text{N}_2\text{O}/\text{N}_2$ ratio (0.11 - 0.34) following an addition of 0-4 mg NO_3^- -N L^{-1} , coupled with a decrease in denitrification efficiency. The mole fractions at all depths (0.01 - 0.03) at sites L1 and L2 were low indicating complete denitrification.

Despite lower N_2O -N concentrations at the L1 and L2 sites, $\text{EF}_{5\text{g}}(2)$ values based on ambient NO_3^- -N levels were at least one order of magnitude higher at the L1 and L2 sites than at the H1 and H2 sites, due to lower NO_3^- -N concentrations at the L1 and L2 sites. The mean $\text{EF}_{5\text{g}}(2)$ values across sites (0.0156 ± 0.0067) were similar to the original IPCC default value of 0.015 (IPCC, 1997) but were lower than the latest IPCC default value of 0.0025 (IPCC, 2006). Our results were of a similar order of magnitude to the results reported by Weymann et al. (2008) (0.00058-0.01065) and Jacinthe et al. (1998) (0.00026-0.0370) but were higher than those reported by Weller et al. (1994) (0.0065-0.0087). The IPCC methodology does not consider that groundwater N_2O is the result of simultaneous production and reduction reactions (Well et al., 2005) and does not take into account that such transformations complicate the relationship between N_2O concentration in groundwater and actual indirect N_2O emissions (Holl et al., 2005). The $\text{EF}_{5\text{g}}(1)$ values calculated following the method of Weymann et al. (2008) (Table 2) varied less among sites and were lower than the IPCC ambient NO_3^- -N based methodology, $\text{EF}_{5\text{g}}(2)$. This approach considers total initial N (Well and Butterbach-Bahl, 2010) rather than just NO_3^- -N. The farm scale N balance approach (Table 2) showed two important factors related to indirect N_2O emissions and $\text{EF}_{5\text{g}}$ estimation. First, this approach suggests that) the IPCC (2006) default leaching value of 30% of N input varies strongly with landscape setting, i.e. we observed . 12, 7, 38 and 23% leached at the L1, L2, H1 and H2, respectively. Second, the farm scale N balance approach suggests that $\text{EF}_{5\text{g}}$ should include initial N losses as well as ambient NO_3^- -N, because ambient NO_3^- -N does not represent the total amount of N leached from surface soil, which also contributes to N_2O production.

4.4 Nitrate removal capacity across sites

Initial N loadings and the amount of N leached to groundwater among our sites varied with local hydrogeochemical conditions and land use. The H2 site, which was intensively grazed permanent grassland for dairy production had higher NO_3^- -N accumulation in groundwater than the other sites due to a deeper GWT, higher permeability and low potential for NO_3^- -N reduction. A thicker unsaturated zone can provide a favourable environment for organic N and NH_4^+ to be converted to NO_3^- -N, which can subsequently leach to groundwater. The L2 site, which was grazed clover/grass grassland with low inorganic N application, had the lowest NO_3^- -N accumulation in groundwater (Table 2). There was significant NO_3^- -N reduction via denitrification, and low NO_3^- -N accumulation in groundwater at both the L1 and L2 sites. The low initial N at L1 and L2 could be due to elevated denitrification in the topsoil (surface soils), which we did not quantify. At the H1 and H2 sites soil surface denitrification was likely to be low due to unfavourable environmental conditions. The RP showed how much initial N was transformed to N_2O and excess N_2 in groundwater. RP was high at the L1 (73%) and L2 (41%) sites and these two sites can be considered to be denitrification ‘hot spots’ at the landscape scale. The other two sites (H1 and H2) had comparatively low RP (8 and 4%) resulting in high net NO_3^- -N concentrations. Toda et al. (2002) estimated NO_3^- -N reduction of 20% in shallow groundwater (4 m bgl) of a coastal agricultural area in Japan and Weymann et al. (2008) reported median values for RP between 0.33 - 0.68.

4.5 Abundances of denitrifier functional genes in groundwater

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) and at great depths in aquifers (Nielsen et al., 2006), but their

expression requires favourable environmental conditions. Our results are in agreement with Cavigelli and Robertson (2000) who also found that the abundance of denitrifying genes was similar among wells and sites but that gene expression was controlled by hydrogeochemical conditions which in turn controlled the denitrification process.

5. Conclusions

Identification of areas with low or high denitrification is required for assessment of the risk of NO_3^- delivery to surface water and indirect N_2O emissions to the atmosphere. Our results suggest that there is marked variability in groundwater denitrification and N_2O dynamics that is strongly controlled by hydrologic (e.g. permeability, changes in groundwater table depth etc.) and geochemical (DO, Eh, DOC, iron sulphide/manganese sulphide and NO_3^- -N) characteristics. We observed high levels of NO_3^- reduction (from 41 to 73%) where DO and Eh were low ($\text{DO} < 2.0 \text{ mg L}^{-1}$; $\text{Eh} \pm 100 \text{ mV}$). Hydrologic and geochemical factors appear to be more important than biological controls, as denitrifier functional genes were ubiquitous. Subsurface denitrification potential was found to extend from shallow groundwater to considerable depth. Our results suggest that information on hydrologic and geochemical characteristics can be used to predict areas of high and low denitrification in Irish landscapes.

Groundwater has the potential to be an important source of indirect N_2O emissions. Groundwater with high DO and Eh conditions ($\text{DO} > 6.0 \text{ mg L}^{-1}$; $\text{Eh} > 150 \text{ mV}$) had high N_2O -N/(N_2O -N+excess N_2 -N) ratios. The current IPCC methodology being used to estimate the $\text{EF}_{5\text{g}}$, should be updated. In our L sites, nitrogen leaching was much lower (7-12%) than the IPCC default value (30%) suggesting that leaching values can and should be adjusted based on information about site hydrologic characteristics. Our results also suggest that the IPCC

methodology should consider the N that leaches from surface soil as well as the ambient NO_3^- in groundwater as both contribute to N_2O emissions from groundwater.

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References

- Askew, F.E., Smith, R.K., 2005a. Inorganic non-metallic constituents, in: Standard Methods for the Examination of Waters and Waste Water. D.A. Eaton, L.S. Clesceri, E.W. Rice, A.E. Greensberg, (Eds.), ISBN 0-87553-047-8. ISBN 0-87553-047-8, American Public Health Association, 800 1 street, NW Washington, DC, pp. 2001-3710.
- Askew, E.F., Smith, R.K., 2005b. Inorganic and non-metallic constituents: persulfate method for simultaneous determination of total nitrogen and total phosphate, in: Standard Methods for the Examination of Water and Wastewater, Eaton, A.D., Clesceri, L.S., Rice, W.E. and Greenberg, A.E. (Eds.), ISBN 0-87553-047-8. American Public Health Association, Washington DC. pp. 160 – 162.

- Baily, A., Rock, L., Watson, C.J., Fenton, O., 2011. Spatial and temporal variations in groundwater nitrate at an intensive dairy farm in south-east Ireland: Insights from stable isotope data. *Agric. Ecosyst. Environ.* 144, 308-318.
- Barrett, M., Jahangir, M.M.R., Cardenas, L., Khalil, M.I., Johnston, P., Richards, K.G., O'Flaherty, V., 2010. Quantitative molecular biology and gas flux measurements demonstrate soil treatment and depth effects on the distribution and activity of denitrifiers. Abstracts, B43F-06, AGU, San Francisco, California, USA.
- Beauchamp, E.G., Trevors, J.T., Paul, J.W., 1989. Carbon sources for bacterial denitrification. *Adv. Soil Sci.* 10, 113-142.
- Böhlke, J.K., 2002. Groundwater recharge and agricultural contamination. *Hydrogeol. J.* 10, 153-179.
- Böhlke, J.K., Verstraeten, I.M., Kraemer, T.F., 2007. Effects of surface-water irrigation on sources, fluxes, and residence times of water, nitrate, and uranium in an alluvial aquifer. *Appl. Geochem.* 22, 152-174.
- Bouwer, H., Rice, R.C., 1976. A slug test for determining hydraulic conductivity of unconfined aquifers with completely or partially penetrating wells. *Water Resour. Res.* 12, 423-428.
- Boyer, E.W., Richard, B.A., William, J.P., Li, C., Butterbach-bahl, K., Donner, S.D., Skaggs, R.W., Del Grosso, S.T., 2006. Modelling denitrification in terrestrial and aquatic ecosystems at regional scales. *Ecol. Appl.* 16(6), 2123-2142.
- Brady, N.C., Weil, R.R., 2002. The nature and properties of soils. Thirteenth ed. Prentice Hall, New Jersey.
- Carter, J.P., Hsiao, Y.H., Spiro, S., Richardson, D.J., 1995. Soil and sediment bacteria capable of aerobic nitrate respiration. *Appl. Environ. Microbiol.* 61(8), 2852-2858.

- Cavigelli, M.A., Robertson, G.P., 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecol.* 81, 1402-1414.
- Clough, T.J., Addy, K., Kellogg, D.Q., Nowicki, B.L., Gold, A.J., Groffman, P.M., 2007. Dynamics of nitrous oxide in groundwater at the aquatic-terrestrial interface. *Global Change Biol.* 13, 1528-1537.
- Conry, M.J., 2006. Carlow granite: Years of history written in stone. Chapelstown Press Ltd., Carlow, Ireland.
- Curie, F., Ducharme, A., Sebilo, M., Bendjoudi, H., 2009. Denitrification in a hyporheic riparian zone controlled by river regulation in the Seine river basin (France). *Hydrol. Process.* 23, 655-663.
- Daly, D., 2005. The characterisations and analysis of Ireland's river basin districts: groundwater aspects, in: Proceedings of International Association of Hydrogeologists (Irish Group) 25th anniversary seminar, Tullamore, pp. 141-150.
- Davidson, E.A., Matson, P.A., Vitousek, P.M., Riley, R., Dunkin, K., Garcia-Mendez, G., Mass, J.M., 1993. Process regulating soil emission of NO and N₂O in a seasonally dry tropical soil. *Ecol.* 74, 130-139.
- Davidson, E.A., Seitzinger, S., 2006. The enigma of progress in denitrification research. *Ecol. Appls.* 16(6), 2057-2063.
- Deurer, M., Von der Heide, C., Bottcher, J., Duijnisveld, W.H.M., Weymann, D., Well, R., 2008. The dynamic of N₂O near groundwater table and transfer of N₂O into the unsaturated zone: Case study from a sandy aquifer in Germany. *Catena.* 72, 362-373.
- Ehhalt, D., Prather, M., Dentener, F., Derwent, R., Dlugokencky, E., Holland, E., Laksen, I., Katima, J., Kirchhoff, V., Matson, P., Midgley, P., Wang, M., 2001. Atmospheric

chemistry and greenhouse gases, in: Houghton J.T. et al. (Eds.), *Climate change 2001: The scientific basis*. Cambridge Univ. Press, Cambridge, pp. 239–288.

Fitzsimon, V.P., Misstear, B.D.R., 2006. Estimating groundwater recharge through tills: a sensitivity analysis of soil moisture budgets and till properties in Ireland. *Hydrogeol. J.* 14, 548-561.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vorosmarty, C.J., 2004. Nitrogen cycles: past, present and future. *Biogeochem.* 70, 153-226.

Hammersley, M.R., Howes, B.L., 2002. Control of denitrification in a septage-treating artificial wetland: the dual role of particulate organic carbon. *Water Res.* 30(12), 3025-3031.

Heaton, T.H.E., Vogel, J.C., 1981. Excess air in groundwater. *J. Hydrol.* 50, 201-216.

Hiscock, K.M., Bateman, A.S., Muhlherr, I.H., Fukada, T., Dennis, P.F., 2003. Indirect emissions of nitrous oxide from regional aquifers in the United Kingdom. *Environ. Sci, Technol.* 37, 3507-3512.

Holl, B.S., Jungkunst, H.F., Fiedler, F., Stahr, K., 2005. Indirect nitrous oxide emission from a nitrogen saturated spruce forest and general accuracy of the IPCC methodology. *Atmosph. Environ.* 39, 5959-5970.

Intergovernmental Panel on Climate Change (IPCC), 1997. Revised 1996 IPCC guidelines for national greenhouse gas inventories, Reference manual, Vol. 3, Organisation for Economic Cooperation and Development, Paris.

- Intergovernmental Panel on Climate Change (IPCC), 2006. N₂O emissions from managed soils, and CO₂ emissions from lime and urea application, in: Volume 4, Chapter II: Agriculture, Forestry and Other Land Use; 2006 IPCC Guidelines for National Greenhouse Gas Inventories (Eds.), Eggleston, H.S., Buendia, I., Miwa, K., Ngara, T., Takabe, K., IGES, Hayama, Japan. pp. 11.1-11.54
- Jacinthe, P.A., Groffman, P.M., Gold, A.J., Mosier, A., 1998. Patchiness in microbial nitrogen transformations in groundwater in a riparian forest. J. Environ. Qual. 27, 156-164.
- Jahangir, M.M.R., Johnston, P., Khalil, M.I., Groffman, P.M., Richards, K.G., 2013. Quantification of *in-situ* denitrification rates in groundwater below an arable and a grassland systems. Water Air Soil Pollut. (submitted).
- Kana, T.M., Darkangelo, C., Hunt, M.D., Oldham, D., Bennet, J.B., Cornwell, J.C., 1994. A membrane inlet mass spectrometer for rapid high precision determination of N₂, O₂, and Ar in environmental water samples. Anal. Chem. 66, 4166-4170.
- Kolle, W., Strebel, O., Bottcher, J., 1985. Formation of sulphate by microbial denitrification in a reducing aquifer. Water Suppl. 3, 35-40.
- Konrad, C., 2007. Methoden zur Bestimmung des Umsatzes von Stickstoff, dargestellt für drei pleistozane Grundwasserleiter Nord-deutschlands, PhD thesis, Dresden Univ. Technol., Germany, p.157.
- Linne, Von Berg, K.H., Bothe, H., 1992. The distribution of denitrifying bacteria in soil monitored by DNA-probing. FEMS Microbiol. Ecol. 86, 331-340.
- Magalhaes, C., Moreira, R., Wiebe, W.J., Bordalo, A.A., 2003. Salinity and inorganic nitrogen effects on nitrification and denitrification rates in inter-tidal sediments and rocky biofilms: Douro River Estuary, Portugal, in: Bruen, M. (Eds.), Proceedings of

- the 7th International Specialised IWA Conference on Diffuse Pollution and Basin Management, Dublin, pp. 6.73–6.79.
- Maître, V., Cpsandey, A.C., Desagher, E., Parriaux, A., 2003. Effectiveness of groundwater nitrate removal in a river riparian area: the importance of hydrogeological conditions. *J. Hydrol.* 278, 76-93.
- McClain, M.E., Boyer, E.W., Dent, C.L., Gergel, S.E., Grimm, N.B., Groffman, P.M., Hart, S.C., Harvey, J.W., Johnston, C.A., Mayorga, E., McDowell, W.H., Pinay, G., 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosysts.* 6, 301-312.
- Neilsen, M.E., Fisk, M.R., Istok, J.D., Pedersen, K., 2006. Microbial nitrate respiration of lactate at *in-situ* conditions in groundwater from a granitic aquifer situated 450 m underground. *Geobiol.* 4(1), 43-52.
- Rabalais, N.N., 2002. Nitrogen in aquatic ecosystems. *Ambio.* 31(2), 102-112.
- Reay, D., Smith, K.A., Edwards, A.C., 2003. Nitrous oxide emission from agricultural drainage waters. *Global Change Biol.* 9, 195-203.
- Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.S.N., Bemment, C.D., 2008. Nitrate attenuation in groundwater: A review of biogeochemical controlling processes. *Water Res.* 42, 4215-4232.
- Rust, C.M., Aelion, C.M., Flora, J.R.V., 2000. Control of pH during denitrification in subsurface sediment microcosms using encapsulated phosphate buffer. *Water Res.* 34(5), 1447-1454.
- SAS Institute Inc. SAS/STAT 9.1 User's Guide. Cary, NC; 2009.

- Sawamoto, T., Kusa, K., Hu, R., Hatano, R., 2003. Dissolved N₂O, CH₄ and CO₂ in pipe drainage, seepage and stream water in a livestock farm in Hokkaido, Japan. *Soil Sci. Plant Nutr.* 48, 433-439.
- Schulte, R.P.O., Diamond, J., Finkle, K., Holden, N.M., Brereton, A.J., 2005. Predicting the soil moisture conditions of Irish grasslands. *Irish J. Agril. Food Res.* 44, 95-110.
- Seiler, K.P., Vomberg, I., 2005. Denitrification in a karst aquifer with matrix porosity, in: Razowska-Jaworek, L., Sadurski, A. (Eds.), *Nitrate in groundwater*. International Association of Hydrogeologists Selected Papers 5, Balkema, Leiden.
- Toda, H., Mochizuki, Y., Kawanishi, T., Kawashima, H., 2002. Denitrification in shallow groundwater in a coastal agricultural area in Japan. *Nutr. Cycl. Agroecosyst.* 63, 167-173.
- Townsend, A.R., Davidson, E.A., 2006. Denitrification across landscapes and waterscapes. *Ecol. Appl.* 16(6), 2055-2056.
- Townsend, A.R., Howarth, R.W., Bazzaz, F.A., Booth, M.S., Cleveland, C.C., Collinge, S.K., Dobson, A.P., Epstein, P.R., Holland, E.A., Keeny, D.R., Mallin, M.A., Rogers, C.A., Wayne, P., Wolfe, A.H., 2003. Human health effects of a changing global nitrogen cycle. *Front. Ecol. Environ.* 5, 240-246.
- USEPA, 1996. Low stress purging and sampling procedure for the collection of groundwater samples from monitoring wells. Quality Assurance Unit, USEPA Region 1, 11 Technology Drive, North Chelmsford, MA 01863, pp. 1-30.
- Von der Heide, C., Bottcher, J., Deurer, M., Duijnisveld, W.H.M., Weymann, D., Well, R., 2009. Estimation of indirect nitrous oxide emissions from a shallow aquifer in northern Germany. *J. Environ. Qual.* 38, 2116-2171.

- Von der Heide, C., Bottcher, J., Deurer, M., Weymann, D., Well, R., Duijnsveld, W.H.M., 2008. Spatial variability of N₂O concentrations and of denitrification related factors in the surficial groundwater of a catchment in northern Germany. *J. Hydrol.* 360, 230-241.
- Weiss, R.F., The solubility of nitrogen, oxygen and argon in water and seawater. *Deep- Sea Res.* 17, 721-735.
- Well, R., Butterbach-Bahl, K., 2010. Indirect emissions of nitrous oxide from nitrogen deposition and leaching of agricultural nitrogen, in: K, Smith (Eds.), *Nitrous oxide and climate change*, London, U.K. pp. 162-189.
- Well, R., Hooper, H., Mehranfar, O., Meyer, K., 2005. Denitrification in the saturated zone of hydromorphic soils – laboratory measurement, regulating factors and stochastic modelling. *Soil Biol. Biochem.* 37, 1822–1836.
- Weller, D.E., Correll, D.L., Jordan, T.E., 1994. Denitrification in riparian forests receiving agricultural discharges, in: Mitsch, W.J. (Eds.), *Global Wetlands: Old World and New*, pp. 117-131.
- Weymann, D., Geistlinger, H., Well, R., Von der Heide, C., Flessa, H., 2010. Kinetics of N₂O production and reduction in a nitrate-contaminated aquifer inferred from laboratory incubation experiments. *Biogeosci.* 7, 1953-1972.
- Weymann, D., Well, R., Flessa, H., Von der Heide, C., Deurer, M., Meyer, K., Konrad, C., Walther, W., 2008. Groundwater N₂O emission factors of nitrate-contaminated aquifers as derived from denitrification progress and N₂O accumulation. *Biogeosci. Discuss.* 5, 1215-1226.

- Weymann, D., Well, R., Von der Heide, C., Flessa, H., Bottcher, J., Duijnsveld, W.H.M.,
2009. Recovery of groundwater N₂O at the soil surface and its contribution to total
N₂O emissions. *Nutr. Cycl. Agroecosyst.* 85, 299-312.
- World Health Organization (WHO)., 2004. *Guidelines for Drinking Water Quality*, 3rd ed.
WHO, Geneva.

Fig. 1 Mean groundwater N_2O ($\pm\text{SE}$) concentrations in subsoil, the interface and bedrock zones at four sites; L1, L2, H1 and H2 from February 2009 to January 2011

Fig. 2 Mean groundwater excess $\text{N}_2\text{-N}$ ($\pm\text{SE}$) concentrations in subsoil, the interface and bedrock zones at four sites; L1, L2, H1 and H2 from February 2009 to January 2011

Fig. 3 N_2O mole fractions vs. excess N_2 using mean ($\pm \text{SE}$) values from the whole study period ($n=36$)

Fig. 4 Groundwater $\text{NO}_3^- \text{-N}$ concentrations versus (a) $\text{RP} \{(\text{N}_2\text{O-N} + \text{excess N}_2\text{-N})/\text{Total N}_{\text{ini}}\}$, (b) TDN ($\text{N}_2\text{O} + \text{excess N}_2$) and (c) Excess N_2 using mean ($\pm \text{SE}$) values from the whole study period ($n=36$)

Fig. 5 Relative abundances of denitrifier functional genes (mean $\pm \text{SE}$); nitrite reductase *nir* (comprised of *nirK* and *nirS*) and nitrous oxide reductase *nosZ* in subsoil, interface and bedrock zones at the (a) L1, (b) L2, (c) H1 and (d) H2 sites. There were no significant differences between sites and depths.

Table 1 Hydrogeochemical characteristics of study sites at the beginning of study

Table 2 Groundwater initial nitrate and total nitrate removal capacity, N₂O mole fraction and emission factors across sites and depths

Table 3 Estimated coefficients of physico-chemical properties selected as significant explanatory variables using a stepwise procedure for models of denitrification products and ratios (n=864)