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Quantification of *in situ* denitrification rates in groundwater below an arable and a grassland system

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Abstract

Understanding denitrification rates in groundwater ecosystems can help predict where agricultural reactive nitrogen (N) contributes to environmental degradation. *In situ* groundwater denitrification rates were determined in subsoil, at the bedrock-interface and in bedrock at two sites, grassland and arable, using an in situ 'push-pull' method with ^{15}N labelled nitrate (NO_3^--N). Measured groundwater denitrification rates ranged from 1.3 to 469.5 µg N $kg^{-1}d^{-1}$. Exceptionally high denitrification rates observed at the bedrock-interface at grassland site (470±152µg N $kg^{-1}d^{-1}$; SE, standard error) suggest that deep groundwater can serve as substantial hotspots for NO_3^--N removal. However, denitrification rates at the other locations were low and may not substantially reduce NO_3^--N delivery to surface waters. Denitrification rates were negatively correlated with ambient dissolved oxygen (DO), redox potential (Eh), k_s and NO_3^- (all p-values $p<0.01$) and positively correlated with SO_4^{2-} ($p<0.05$). Higher mean $N_2O/(N_2O+N_2)$ ratios at arable (0.28) site than the grassland (0.10) revealed that arable site has higher potential to indirect N_2O emissions. Identification of areas with high and low denitrification and related site parameters can be a tool to manage agricultural N to safeguard the environment.

Key words: Denitrification, ^{15}N-enrichment, ^{15}N-N_2O, ^{15}N-N_2, groundwater, N_2O mole fraction

1. Introduction

The nitrogen (N) cascade is an increasingly important global issue with multiple impacts on terrestrial, aquatic and atmospheric environments (Galloway et al., 2008). The high rates of N deposition result in N saturation in agricultural land causing high nitrate (NO_3^--N) delivery to groundwater which is of concern with result to global environment and human

health (Organisation of Economic Co-operation and Development, 2009). In Ireland, groundwater beneath some agricultural systems is contaminated with NO_3^- and this also contributed to the eutrophication of estuarine and near coastal waters (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, 2002).

The biogeochemical process, denitrification, is the principal process which converts the NO_3^--N to nitrous oxide (N_2O) and dinitrogen (N_2) gas (Rivett et al., 2008). The intermediate product N_2O is a potent greenhouse gas with global warming potential 298 over a 100 year time period. Indirect N_2O emissions resulting from N leaching into associated groundwater are an important but poorly understood component of global N_2O budget (Clough et al., 2007). The quantity of the end product of denitrification process, N_2, is by far the largest uncertainty of the N cycle at all scales (Galloway et al., 2004). Therefore, narrowing this uncertainty is critical if improvements are to be made in global N_2O and N_2 budgets. Quantification of $N_2O/(N_2O+N_2)$ ratios in groundwater would help refine greenhouse gas inventories and provide insights into the relative contribution of denitrification to environmentally benign N_2 production.

As denitrifiers are reported to be ubiquitous in shallow to deep groundwaters (Linne von Berg and Bothe, 1992; Francis et al., 1989) the availability of energy sources and suitability of the hydrogeological environments for denitrifiers need to be investigated. Barrett et al. (2013) quantified denitrification genes in four Irish aquifers (up to 50 m), including the two sites in the current study. They found similar concentrations of denitrification genes across sites and piezometer depth. Therefore optimum hydrogeochemical conditions for microbial

denitrification can help biodegradation of NO_3^--N (ITRC, 2002). Analysis of dissolved N_2O and N_2 in groundwaters from subsoil (5 m), bedrock-interface (12 m) and bedrock (22 m) in Ireland underlined that denitrification can be an important NO_3^- removal pathway across shallow to deep groundwaters (Jahangir et al., 2012a). However, in groundwater denitrification studies it is often unclear if the denitrification products are produced *in situ* or if they have been leached from surface soils (Groffman et al., 1998). Application of *in situ* remediation to any contaminant and site is gaining wide acceptance as viable and economic technology (ITRC, 2002). However, the denitrification process in groundwater is very difficult to measure, and existing methods used to measure denitrification are problematic for a variety of reasons (*e.g.*, high background N_2, degassing of samples and physical attenuation) (Groffman et al., 2006). The *in situ* NO_3^- push-pull method has been used to determine denitrification in shallow groundwater (<3 m) (Addy et al., 2002; Kellogg et al., 2005). Istok et al. (1997) used the push-pull method for measuring groundwater denitrification in a sand and gravel aquifer at a depth of approximately 10 m. However, in the deep groundwater zones it can be more challenging due to the complex hydrogeological settings e.g., high permeability or preferential flow through fracture in bedrock resulting in high physical attenuation (Buss et al., 2005). In this study, the push-pull method was extended from shallow to deep groundwaters (up to 22 m) to quantify denitrification rates. The objectives of this study were to (a) assess application of the 'push-pull' method in deep groundwaters; (b) determine *in situ* denitrification rates in shallow to deep groundwaters; (c) quantify the N_2O mole fractions, $N_2O/(N_2O+N_2)$; and (d) identify factors controlling the observed spatial trends of denitrification rates.

2. Materials and Methods

115 2.1 Experimental site characteristics

116 The *in situ* NO_3^- push-pull method was used at two groundwater monitoring sites in
117 Southeastern Ireland (Figure 1). The sites were: Johnstown Castle (52° 17' 30" N, 6° 29' 50"
118 W), a poorly drained intensively managed grazed (35 years) grassland, and Oak Park (52° 51'
119 43" N, 6° 54' 53" W), a well drained arable land with spring barley-cover crop rotation (10
120 years). Both sites receive approximately 312 and 150 kg N ha^{-1} as organic and inorganic
121 forms of N, resulting in N surpluses of 243 and 75 kg N ha^{-1}, respectively. The grassland site
122 comprises poorly drained top soils overlying clayey subsoils inter-mixed with sands and
123 gravels followed by ordovician sediments, sandstone and shale at 10 m. At the arable site, soil
124 profile comprises well drained top soil overlying subsoils of sands, gravel and inter-bedded
125 clay band followed by grey limestone at 10 m (Figure 2). Three distinct water tables were
126 encountered on each of the sites and these were specifically targeted with piezometers (Figure
127 2). The aquifer beneath the grassland site is poorly productive, with a shallow perched water
128 table but has had elevated NO_3-N concentrations reported (Fenton et al., 2009). At the arable
129 site there had a productive sand and gravel aquifer overlying a productive limestone aquifer,
130 both of which were vulnerable to NO_3^--N pollution as previously described by Premrov et al.
131 (2012). The hydrologic and geochemical properties of the sites were presented in Table 1. The
132 grass and arable sites represent approximately 62 and 37% of Irish soil types and 21 and 71%
133 of bedrock types, respectively.

134

135 2.2 *In situ* Push-Pull Method

136 We adapted the *in situ* push–pull method of Addy et al. (2002) and Kellogg et al. (2005)
137 to estimate denitrification rates in shallow (5 m bgl, below ground level) to deep (12-22 m
138 bgl) groundwaters. Groundwater wells (PVC with 0.05 m i. d.; 2 m screen section) were

placed along groundwater flow paths at three depths to target samples in (S) subsoil (5 m bgl), (I) bedrock-interface (12 m bgl) and (B) bedrock (22 m bgl). The push-pull method comprised two steps: (1) the push-pull pre-test and (2) the NO_3^- push-pull test. The study (pre-test and NO_3^- push-pull test) was conducted during October -December 2010.

2.4 *In Situ* Push-Pull Pre-test

In Situ Push-Pull Pre-test was conducted to gain insights into balancing high recovery of the plume with sufficient time *in situ* for microbial denitrification to occur at detectable levels. Twenty litres of groundwater was collected from each well, amended with a conservative tracer bromide (Br^-; 20 mg L^{-1}) and pushed into the same well (at least one well per depth per site) using a peristaltic pump (Model 410, Solinst Canada Ltd.). The dosing solution amended with Br^- was sampled during the push phase to obtain the undiluted concentration of Br^-. The push-pull pre-test was conducted repeatedly with initial incubation for a 12-h period and then lowered to 3-h with the estimation of corresponding recoveries of Br^-. An incubation period less than 3-h was not attempted because of the concern that there would be low detection of denitrification gases in the subsequent NO_3^- push-pull test, particularly in deep bedrock. After the incubation period groundwater (twice the dosing volume), pulled up using a Grundfos pump (Model MP1, Grundfos, Fresno, CA, USA) taking samples at 2 L intervals, was analysed for the Br^- recovery at each sample intervals. A peristaltic pump was not used to pump water because of its inability to pump water from depths greater than 6 m bgl. Groundwater injection and pumping back were conducted slowly to prevent changes in hydraulic gradient around the well. After 1 week, groundwater in the pre-tested wells 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 or before conducting the in situ NO_3^- push–pull test.

The injected volume of water was sufficient to fill approximately 270 to 1000 kg of aquifer materials (bulk density= 1650 - 2500 kg m^{-3}, porosity = 0.03 - 0.12) after correcting for the sand and gravel pack around the well. The total amount of aquifer materials covered by the solution was calculated using the Eqn.1 below:

$$Mt = [\frac{(Vt - Vg)}{Porosity\ of\ aquifer}] * Bd \qquad \text{(Eqn. 1)}$$

where Mt is the total mass of aquifer materials (kg), Vt is the total volume of solution (m^3), Vg is the volume of gravel pack (m^3), and Bd is the bulk density (kg m^{-3}).

2.5 *In Situ* ^{15}N-NO_3^- Push–Pull Experiment

In situ NO_3^- push-pull tests were conducted in S, I and B with three replications per depth. Twenty liters groundwater was collected in a carboy from each well and stored in a cold room at 4° C for maximum 2 days. To adjust the dissolved oxygen (DO) back to ambient conditions, groundwater solution was bubbled with sulphur hexafluoride (SF_6; 98.2%, Cryoservice Ltd., Worcester WR4 9RH, UK) while the DO concentration was monitored using a DO probe (Multi 340i/SET, WTW, Germany). The SF_6 can also serve as a conservative tracer. The dosing solution was prepared with ambient groundwater, 20 mg L^{-1} Br^- as KBr and 20 mg N L^{-1} as isotopically enriched KNO_3 (50 atom% ^{15}N-KNO_3; purity 99%). The carboy with dosing solution was capped and its headspace was filled with the SF_6 gas. The SF_6 headspace was maintained with same pressure while connected to a SF_6 gas cylinder (carried to field) during the injection of the dosing solution. The dosing solution was injected into the respective well over the course of 1- 2-h (depending on the permeability, Table 1; and hydraulic gradient, data not shown) with a peristaltic pump with a Teflon outlet

186 at a very low rate (10 to 15 L h^{-1}). Samples were collected for DO, SF_6, Br^- and other
187 dissolved gases and hydrochemistry during the middle of the injection phase.

188 The incubation period was defined as the length of time between the end of the push phase
189 and the start of the pull phase since the plume core would consist mostly of the later injected
190 groundwater. The incubation period for the dosing solution was set at 6-h, based on pre-test
191 results so that there was substantial plume recovery and sufficient incubation time. After the
192 incubation period, groundwater was pumped back from the well slowly (10 to 15 L h^{-1}) using
193 a Grundfos pump with a Teflon outlet. As the injected volume was pumped, such samples
194 were taken using a syringe attached to an air-tight sampling apparatus made of stainless steel
195 tubing connected to the outlet of the Grundfos pump. Groundwater samples (120 ml) were
196 injected into an evacuated serum bottle (160 ml) and the headspace (40 ml) was filled with
197 high-purity helium gas (*He*: water ratio = 1: 3; v/v), and then submerged under water in a
198 polystyrene box and stored at 4°C. For each well, conservative tracers (Br^- and SF_6)
199 recoveries were estimated as *C/Co*; where C was the tracer's concentrations in the pulled
200 groundwater following incubation and *Co* was the tracer's concentrations in the original
201 pushed groundwater (Freeze and Cherry, 1979).

202

203 2.6 Dissolved Gas Analysis

204 Groundwater dissolved gases (N_2O, N_2 and SF_6) in ambient, pushed, and pulled samples
205 were extracted using the phase equilibration headspace extraction technique, with *He* filling
206 the headspace (Lemon, 1981; Davidson and Firestone, 1988) in the lab on the same day of
207 sample collection. Groundwater samples collected in the serum bottles were shaken for 5 min
208 on a Gyrotory shaker (Model G-10, New Bruns- wick Scientific Co., USA) and left for a
209 standing period of 30 min. Headspace samples were then taken for the analysis of SF_6, N_2O

and N_2 concentrations and the ^{15}N enrichment of N_2O and N_2 in 12 ml exetainers (Labco Inc. Wycomb, UK) after injecting additional 12 ml high purity *He*. The N_2O and SF_6 gases were analysed on a gas chromatograph (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. The GC had a Porapak-Q column (80-100 MESH), 3.7 m x 1/8" x 2.0 mm. Concentrations and ^{15}N enrichment of N_2O and N_2 were determined on a dual-inlet isotope ratio mass spectrometer (Stable Isotope Facility, UC Davis, Davis, CA) as described by Mosier and Schimel (1993).

2.7 Calculations of Denitrification Rate

Dissolved N_2O and N_2 concentrations were calculated using the three highest recovery values within sample replicates (Harrison et al., 2011). The masses of dissolved N_2O–N and N_2 gases (μg) were calculated from the headspace extraction samples using equations and constants provided by Tiedje (1982) and Mosier and Klemedtsson (1994). The total mass of N_2O–N or N_2 was then 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_2O-N and N_2 to pushed NO_3^- –N atom %, both corrected for ambient atom %). Gas production rates for $^{15}N_2O$–N and $^{15}N_2$-N were expressed as μg N kg^{-1} soil d^{-1} as below:

$$\text{Rates } \mu g\ N kg^{-1} d^{-1} = \frac{Total\ mass\ of\ ^{15}N_2O - N\ and\ ^{15}N_2 - N\ per\ volume\ of\ water\ pulled}{Dry\ mass\ of\ soil\ per\ volume\ of\ water * incubation\ period\ pulled} \quad \text{(Eqn. 2)}$$

Mass of aquifer materials was calculated for individual depths at each site. Total denitrification rates were the sum of $^{15}N_2O$–N and $^{15}N_2$ generation rates. All samples used in denitrification calculations contained at least 8 mg L^{-1} NO_3^- –N to ensure that calculated denitrification rate estimates were not limited by the amount of NO_3^--N available (Schipper and Vojvodic-Vukovic, 1998).

234 2.8 Hydrological and geochemical analyses

235 Groundwater permeability (k_s) was estimated using the slug test method (Bouwer and
236 Rice, 1976) with 20 seconds for the initial linear point to eliminate the drainage in the gravel
237 pack. Groundwater table (GWT) depth was measured using an electrical dip meter. Samples
238 for DO were collected in a 12 ml exetainer (Labco Ltd, Wycombe, UK), after slowly
239 overflowing approximately 10 ml excess water and closed immediately using double septum
240 (butyl rubber + Teflon) stopper. Samples were submerged under water in a polystyrene box,
241 stored at 4ºC and analysed within one week. DO was measured by membrane inlet mass
242 spectrometry (MIMS) (Kana et al., 1994). Groundwater pH, electrical conductivity (EC) and
243 redox-potential (Eh) were measured using a multiparameter probe (Troll 19500, In Situ Inc.
244 USA). Groundwater was analysed for NO_3^--N and Br^- on DX-120 ion chromatography
245 (Metrohm UK Ltd.). The DOC was analysed using Total Organic Carbon Analyser (TOC-V
246 cph/cpn; Shimadzu Corporation, Kyoto, Japan). Groundwater non-metallic ions e. g. total
247 oxidised N, nitrite, NH_4^+ and P; reduced metals e.g. Fe^{2+}, Mn^{2+} and S^{2-} were analyzed with an
248 Aquakem 600 Discrete Analyser (Aquakem 600A, Vantaa, Finland). Groundwater SO_4^{2-}
249 concentration was measured with a turbimetric method (Askew and Smith, 2005).

250

251 2.9 Statistical Analyses

252 The measured denitrification rates were approximately log-normally distributed.
253 Therefore, non-parametric Kruskal-Wallis H tests were performed to determine significant
254 differences in groundwater denitrification rates among depths within each site. After
255 significant differences were observed among depths, Mann–Whitney U tests (Ott, 1993) were
256 performed as a post hoc test to determine which depths were significantly different. Paired t
257 tests (Ott, 1993) were performed to determine significant differences in recovery (*C/Co*)

between Br^- and SF_6. Spearman rank order correlations were performed to determine significant correlations between groundwater denitrification rates and ambient DO, Eh, NO_3^--N, DOC and k_s. All statistical analyses were performed on GenStat (2011). All statistical differences were considered significant at $p<0.05$ level.

3. Results

3.1 Groundwater physico-chemical properties

Groundwater ambient physico-chemical properties related to denitrification differed among sites (Table 1). Mean NO_3^--N concentrations were significantly different between sites ($p<0.001$). Considering the within site differences among various depths, NO_3^--N concentrations were significantly higher ($p<0.01$) in S than at the I and B at grassland site but were similar at arable site. Mean NH_4^+ concentrations were low at both sites, with being 0.14 and 0.02 mg L^{-1} at grassland and arable sites, respectively. Groundwater pH was near neutral in all depths at grassland but was higher at I compared with B at the arable site. Reduced Fe (Fe II) concentrations were higher at grassland than that at arable site (Table 1). Mean groundwater SO_4^{2-} concentrations were significantly higher at arable site than the grassland ($p<0.05$) but were similar between depths at each site. Mean S^{2-} concentrations were similar across sites and depths. Mean DO concentrations were significantly lower at the grassland site than at arable site (Table 1). Mean DOC concentrations were significantly higher at grassland (2.6±0.8 mg L^{-1}), than at the arable site (0.9±0.1 mg L^{-1}) ($p<0.05$). Interestingly, DOC was similar between depths at each site, whereas DO significantly decreased ($p<0.05$) with depth at both sites (Table 1). The C/N ratios were significantly higher at grassland than the arable site (data not shown). Irrespective of depths, C/N ratios ranged 1.2 - 20.5 and 0.10 - 0.14 at grassland and arable sites, respectively. Phosphorous (orthophosphate, PO_4^{3-}) concentrations

were below the detection limit in groundwater at both these study sites (<0.005 mg L^{-1}). The Eh at grassland (25-94 mV) site were lower compared with the arable site (107-178 mV) (Table 1). The arable site had a higher aquifer saturated hydraulic conductivity coupled with a deeper groundwater table than at the grassland (Table 1). Saturated hydraulic conductivity (k_s) increased with the increase in groundwater depth (Table 1).

3.2 Assessment of push-pull method for deep groundwaters

The predetermined k_s value in each piezometer (mean 0.009 m d^{-1} ± 0.002 (standard error, SE) at grassland; mean 0.049 m d^{-1} ± 0.008 at arable) provided an insight into the potential incubation times for push-pull pre-test. However, push-pull pre-test at both sites revealed a significant influence of incubation time on the recovery of tracer (Br^-) injected into the piezometer ($p<0.001$). Reducing the incubation time increased tracer recovery from 9-30% for the 12-h incubation to 30-80% for the 3-h incubation. In the NO_3^- push-pull test, the percentage recovery of the two tracers used (Br and SF_6) were similar ($p>0.05$) to each other. Mean recovery of the Br^- and SF_6 tracers did not differ significantly among groundwater depths within each site but differed between the two sites. Mean Br^- recoveries in the core plume (the first 2-4 L of the pull where recovery is the highest) after a 6-h incubation ranged from 43% in B to 59% in S at grassland and 39% in B to 55% in S at the arable site.

3.3 *In-situ* denitrification rates

Over the short incubation period (6-h), NO_3^- removal via denitrification was detected at both sites. Denitrification rates at grassland site (mean = 163 μg N kg^{-1} d^{-1}±153 (SE) were significantly higher than that at the arable site (mean = 3.9 μg N kg^{-1} d^{-1}±2.0). Among depths within the grassland site (Figure 3a), significantly higher denitrification rates were measured

306 at I (mean = 470 μg N kg^{-1} d^{-1}±111); than S (mean =10.9 μg N kg^{-1} d^{-1}±3.5) or B (mean = 9.2
307 μg N kg^{-1} d^{-1}±2.8). Similarly denitrification rates in the three different depths at the arable site
308 were significantly higher ($p<0.05$) at I (6.4 μg N kg^{-1} d^{-1}±1.8) than S (3.8 μg N kg^{-1} d^{-1}±0.7)
309 or B (1.4 μg N kg^{-1} d^{-1}±0.4) (Figure 3b). Mean denitrification rates were equivalent to a
310 weighted average of 3.92 and 0.09 mg NO_3^--N L^{-1} d^{-1}, respectively at the grassland and arable
311 sites, which accounted for 24.5 and 0.33% of the N input to the land. Denitrification rates
312 individually in the S, I and B at grassland were equivalent to 0.2, 10.3 and 0.3 mg N L^{-1} d^{-1}
313 which accounted for 1, 65 and 2% of the N input, respectively. The coefficient of variations
314 (CV) for denitrification rates between wells was 55, 115 and 109% in the S, I and B,
315 respectively at the grassland and 117, 60 and 47% in S, I and B at the arable site.
316
317 3.4 N_2O mole fraction
318 The $N_2O/(N_2O+ N_2)$ ratios were significantly higher at the arable site (mean = 0.28 ±0.04)
319 than at the grassland site (mean = 0.10±0.02) (Figure 4). Among the three depths, $N_2O/(N_2O+$
320 N_2) ratios were significantly higher in S and I than B at arable site. In contrast, they were
321 lower in S than I and B at the grassland (Figure 4a, 4b). In situ production of environmentally
322 benign N_2 was the dominant end product of denitrification and ranged from 89-93% of the
323 total denitrification gases at the grassland site, whereas at the arable site it ranged from 62-
324 85% of the total denitrification gases.
325
326 3.5 Relationships between denitrification rates and ambient hydrogeochemical conditions
327 Spearman Rank Order correlation between denitrification rates and ambient geochemical
328 properties showed significantly negative correlations between denitrification rates and
329 ambient DO ($r = -0.52$, $p<0.05$), Eh ($r = -0.52$, $p<0.05$), NO_3^--N concentrations ($r = -0.69$,

330 $p<0.01$), and saturated hydraulic conductivity ($r = -0.50$, $p<0.05$). There was no significant
331 correlation observed between denitrification rates and ambient DOC concentrations in
332 groundwater. In addition, denitrification rates showed a positive correlation with reduced Fe
333 (Fe II; $r=0.39$; $p<0.05$), SO_4^{2-} ($r=0.32$; $p<0.05$) and NH_4^+($r=0.33$; $p<0.05$). A conceptual
334 model showing site hydrogeochemistry, groundwater denitrification and NO_3^--N pollution
335 potential was presented in Figure 5.

336

337 **4. Discussion**

338 4.1 Assessment of push-pull method for deep groundwaters

339 Estimation of tracer recovery is very important for quantifying groundwater denitrification
340 rates and to understand the decline in concentrations of denitrification end products by
341 physical processes like advection, dispersion and diffusion. Both Br^- and SF_6, being used in
342 these sites, had similar rates of recovery in the NO_3^- push pull test and indicated that there was
343 no degassing loss of SF_6 during the incubation and sampling. The similarities in the recovery
344 of both tracers also enhance the confidence of estimating groundwater dissolved gas
345 concentrations produced via denitrification during the incubation period. Bromide has been
346 used as a tracer because in groundwater, it does not come in to contact with vegetation, thus
347 uptake by plant is minimized (Richards et al., 2005). However, either of the tracers can be
348 used for investigating groundwater denitrification using the push-pull test. Only Br^- has been
349 used as the conservative tracer in many riparian groundwater NO_3^- studies (Simmons et al.,
350 1992; Nelson et al., 1995; Starr et al., 1996) and other in situ riparian studies (Addy et al.,
351 2002; Clough et al., 2007; Kellogg et al., 2005) have used both Br^- and SF_6 as conservative
352 tracers.

353 Recovery rates in this study (5-22 m bgl) were relatively lower than the push-pull studies
354 by Addy et al. (2002) and Kellogg et al. (2005). Both studies incubated the dosing solutions
355 for variable times e.g., 4 to 24-h (Kellogg et al., 2005) and 5 to 72-h (Addy et al., 2002). Their
356 higher tracer recoveries were found at shallower depths i.e. in 0.65 to 1.25 m and 0.65 to 3 m
357 that provided a maximum recovery of 80 and 70%, respectively. Our tracer recoveries were
358 within the range found by Harrison et al. (2011); 42-54% recovery in summer and 20-26% in
359 winter in two alluvial wetlands with minipiezometer to a depth of 0.5 m and incubated for 4-
360 h. Low tracer recovery in our study is likely due to high advective dispersion and diffusion
361 and low residence time in these aquifers which have sediments with larger and more
362 connected secondary pores or preferential flow path via fracture/fissure (Buss et al., 2005;
363 Misstear et al., 2009). Sedimentary rocks e.g., Ordovician sediments, sandstones in the
364 grassland site and limestones at the arable sites showed increased hydraulic conductivity with
365 depth of aquifers. Solute movement follows piston flow model in subsoil but in bedrock it
366 follows complex pattern of movement because bedrock might have both vertical and
367 horizontal flow paths via fractures developed by glacial movement.

368

369 4.2 Variations in groundwater denitrification rates

370 Denitrification rates were highest at I of the grassland site, higher than observed in the S.
371 Our lower denitrification rates were within the range of shallow groundwater denitrification
372 rates reported by Kellogg et al. (2005) (<1 to 330 ug N kg^{-1} d^{-1}), Addy et al. (2002) (2.1 to
373 123.2 ug N kg^{-1} d^{-1}) and Harrison et al. (2011) (<0.1 to 193 ug N kg^{-1} d^{-1}), but our grassland I
374 high value was higher than reported by these other *in situ* push-pull papers. Higher
375 denitrification rates at I (10 m bgl) are in line with the findings of Weymann et al. (2010)

who, from a laboratory incubation experiment, observed that NO_3^- removal in the autotrophic zone (6.5 to 7.0 m bgl) is much more intensive than shallow zone (1.5 to 4.0 m bgl).

Our results suggest that while denitrification is not ubiquitous in deep groundwaters, it can serve as substantial hotspots for groundwater N removal before its delivery to surface waters. Higher denitrification rates at the I indicate that denitrification is not limited to shallow groundwater, rather it can occur in deep groundwaters. This notion 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. However, underestimation of denitrification rates may also occur 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).

Denitrification rates showed high spatial variability because groundwater hydrogeological properties that control denitrification are heterogeneous. The coefficients of variation of N_2O concentrations between wells within each site, ranged from 55-115 and 47-60% at grassland and arable sites, respectively, and 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), 78-122% (Jahangir et al., 2011), 14-132% (Ishizuka et al., 2005) and in shallow groundwater e.g., 219% (Von der Heide et al., 2008). This variation indicates that denitrification is likely to be an active process, as it is in top soil, of natural NO_3^- reduction in shallow to deep groundwaters. Moreover, high spatial variability of N_2O production is consistent with the high spatial variability of 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. The *in situ* push-pull tests were only conducted during one season because dissolved N_2O and N_2 at the sites were previously observed to be similar throughout the year (Jahangir et al., 2012a).

4.3 Variations in N_2O mole fractions

Higher N_2O mole fractions at the arable site than that at the grassland 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 with those measured in a laboratory incubation of subsoil from the grassland site with values of 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 (Jahangir et al., 2012b). The N_2O mole fraction in this study (0.07-0.38) was comparable with Harrison et al. (2011) who measured $N_2O/(N_2O+N_2)$ ratios of 0.02- 0.21 in 0.5 m bgl in alluvial wetlands using the *in situ* push-pull method. Mean N_2O mole fraction, calculated at each site, implies two possibilities: 1) the 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); or 2) N_2O can further be reduced to N_2 (Weymann et al., 2008). Mean mole fractions 0.02 at grassland to 0.09 at the arable site from monthly measurements over two years (2009-2010) in these wells (Jahangir et al., 2012a) were lower than that of the measurements by *in situ* push-pull test, possibly 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 for higher $N_2O/(N_2O+N_2)$ ratios in the *in situ* study than that of the monitoring results of Jahangir et al. (2012a) could be the addition of NO_3^--N to groundwater by at least 2 times of the ambient concentration, as high NO_3^--N 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

423 fractions, taking into consideration the travel time through aquifers which can take from
424 months to years at these sites (Fenton et al, 2011).
425
426 4.4 Relationships between denitrification and ambient hydrogeochemical conditions
427 The differences in denitrification rates between sites and depths may be explained by their
428 contrasting hydrologic and geochemical conditions (Table 1). The ITRC (2002) highlighted
429 that in situ hydrologic (e.g., groundwater table, k_s and hydraulic gradient), geochemistry (e.g.,
430 Eh, Fe II, DO and TOC) and microorganisms are important factors for bioremediation. The
431 lower k_s at grassland site favoured denitrification. In comparable study Fenton et al. (2009)
432 found that subsoil k_s was negatively related to groundwater N_2/Ar ratio. Fenton et al. (2009)
433 measured saturated hydraulic conductivity in 17 wells in subsoil at grassland site by slug
434 which ranged from 0.001 to 0.016 m d^{-1}. These hydraulic conductivity values were
435 comparable with the range of present study. Fitzsimons and Misstear (2006) reported the
436 hydraulic conductivity values of some low to moderate permeable tills in Ireland ranging
437 from 0.0004 to 0.009 m d^{-1} which was within the range of the current study at the grassland
438 site. The DO, being comparable in all depths at the grassland site, was lower than the arable
439 site. The low DO and low Eh indicate the higher anaerobiocity of groundwater that could
440 foster denitrification. Rivett et al. (2008) identified DO and electron donor concentrations and
441 availability as the primary factors governing denitrification in groundwater. Böhlke et al.
442 (2007) observed <1.6 mg L^{-1} of DO was required for complete denitrification of NO_3^--N to
443 N_2. The higher DO and Eh at the arable site suggests that *in situ* denitrification may be either
444 very low or zero under these conditions. The observed denitrification rates, though small at
445 the arable site, could be attributed to either deriving from aerobic denitrification (Robertson et
446 al., 1995) or through denitrification occurring in anaerobic microsites (Seitzinger et al., 2006).

447 From groundwater monitoring results of hydrochemistry and dissolved gases (N_2O and excess
448 N_2, called denitrified N_2), higher NO_3^--N and lower N_2O and N_2 concentrations were
449 previously observed at the arable site (Jahangir et al., 2012a) supporting this theory. On the
450 same sites Barrett et al. (2013) observed nir and nosZ abundance (these are the functional
451 genes associated with nitrite and nitrous oxide reductase) of 13.5 -4.6×10^3 and 9.8-18.3×10^2
452 (gene copy conc. L^{-1}), indicating that microbial occurrence is unlikely to be a limiting factor
453 for groundwater denitrification.

454 DOC enhances denitrification by reducing DO through aerobic respiration, releasing CO_2
455 and as an electron donor for denitrifier community. Moreover, DOC was available to shallow
456 groundwater and also the deep groundwater as there was no significant decline in DOC with
457 depth from 5 to 22 m bgl. The lack of any significant correlation between DOC and
458 denitrification rates may be due to the high spatial variabilities in DOC concentration (<1 to
459 >10 mg L^{-1}). In deep groundwaters, however, other electron donors, such as Fe minerals, can
460 be of importance as denitrification rates showed positive correlation with reduced Fe, which
461 was the highest at the I at grassland site. The oxidation of sulphide compounds (bound with
462 Fe) under anaerobic conditions may release Fe (II) or Mn (Kolle et al., 1985). Negative
463 correlations between denitrification and ambient NO_3^- concentration implies that low ambient
464 NO_3^- existed in groundwater wells due to occurrence of natural denitrification process that
465 substantially reduced NO_3^- (Konrad, 2007; Vogel et al., 1981; Weymann et al., 2008). In
466 denitrification process, if reduced S is the electron donor, SO_4^{2-} is formed (Rivett et al., 2008).
467 The positive correlation between groundwater SO_4^{2-} and denitrification rates might be
468 contributed to oxidation of sulphur in anaerobic environment where S^{2-} (reduced S or metal
469 bound S) might be an important electron donor (autotrophic denitrification). The NH_4^+, being
470 observed mainly at grassland, showed positive correlation with denitrification rates because

471 NH_4^+ generation via dissimilatory nitrate reduction to ammonium (DNRA) might have
472 occurred in the anaerobic environment which is a requirement for denitrification.
473
474 **Conclusions**
475 The results of this study show that the push-pull method for groundwater denitrification
476 rates using ^{15}N-enriched NO_3^--N can be used in the deep groundwater systems. Low
477 conservative tracer recovery may have underestimated denitrification estimates. The bedrock-
478 interface at the grassland site with low DO, Eh and high DOC demonstrates that deep
479 groundwater can serve as a 'hot spot' for NO_3^- removal. Even where we observed low
480 denitrification rates at the arable site with high DO, Eh and low DOC, its contribution to
481 indirect N_2O emissions should still be accounted for in global N_2O budgets. The strong
482 correlations between denitrification rates and hydrogeologic conditions suggest that
483 modelling within geographical information systems may help to predict locations with
484 substantial subsurface denitrification rates. These findings show important implications about
485 the natural NO_3^--N attenuation capacity of groundwater beneath intensively managed
486 grassland that reduces the risk of NO_3^--N delivery to the surface waters. In addition, N_2O
487 mole fractions from *in situ* measurements indicated that groundwater denitrification can
488 reduce indirect N_2O emissions to the atmosphere. Therefore, NO_3^--N reduction to N_2O and to
489 N_2, while transported through groundwater to the receptors are simultaneous processes which
490 balance net NO_3^--N delivery to surface waters and indirect N_2O emissions to atmosphere.
491
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682 *Table 1* Ambient hydrologic and hydrochemical properties; values are means ± SE, n=3

Depth	NO_3^--N	DOC‡	Fe (II)	SO_4^{2-}	S^{2-}	DO‡	pH	Eh	GWT	k_s
	….….....		mg L^{-1}	.....				(m V)	(m, bgl)	(m d^{-1})
Grassland										
Subsoil 5 m bgl	4.7±1.6a	1.0±0.1a	12±4a	20.0±1.6a	0.26±0.04a	1.9±0.1a	6.9±0.1a	94±28a	1.8±0.1a	0.008±0.002a
Interface 12 m bgl	2.0±1.8b	3.5±2.3a	48±27b	19.2±1.6a	0.21±0.06a	1.3±0.4b	6.8±0.1a	25±62b	2.9±0.9b	0.024±0.004a
Bedrock 22 m bgl	2.9±1.3b	3.4±2.7a	14±13a	16.4±1.6a	0.24±0.04a	1.6±0.1b	6.8±0.1a	47±43b	3.4±1.0b	0.030±0.005a
Arable land										
Subsoil 5 m bgl	12.8±2.6a	1.1±0.2a	4.4±1.1a	27.2±1.0a	0.17±0.01a	9.5±1.4a	7.8±1.3b	178±60a	4.2±0.2a	0.033±0.006a
Interface 12 m bgl	10.4±0.3a	0.8±0.2a	4.8±0.7a	23.3±1.1a	0.24±0.08a	6.2±1.6b	8.9±1.2a	163±50a	4.6±0.1a	0.053±0.003a
Bedrock 22 m bgl	12.6±2.5a	0.7±0.2a	2.7±1.0a	27.3±0.7a	0.18±0.05a	4.1±1.4b	7.5±0.1b	107±39b	5.1±0.1a	0.123±0.003b

683 ‡DO is dissolved oxygen; DOC is dissolved organic carbon; GWT is groundwater table; k_s is saturated hydraulic conductivity; bgl is below ground level. The same letter
684 within each site does not differ significantly between depths ($p>0.05$)
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692 **List of Figures**

693 *Figure 1* Experimental sites and multilevel well locations; grassland at Johnstown Castle
694 and arable land at Oak Park in South-Eastern Ireland. Receptors are carrying groundwater to
695 the nearby rivers (river 'Kildavin' at grassland and river 'Barrow' at arable land).

696 *Figure 2* Borehole installation cross sections from sites: Johnstown castle (JC) and Oak
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698 properties, details of water table depths and Ks values were summarised in Table 1

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700 at grassland (n=3) and (b), at arable land (n=3)

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702 groundwater at grassland (n=3) and (b), at arable land (n=3)

703 *Figure 5* A conceptual model showing site hydrogeochemistry vs. denitrification and
704 nitrate (NO_3^--N) pollution potential

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