

1



AGRICULTURE AND FOOD DEVELOPMENT AUTHORITY

2

3

4

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

5

6

7

AUTHORS: M.M.R. JAHANGIR, P. JOHNSTON, K. ADDY, M.I. KHALIL, P.M. GROFFMAN, K.G. RICHARDS

8

9

10

11

12

This article is provided by the author(s) and Teagasc T-Stór in accordance with publisher policies.

Please cite the published version.

The correct citation is available in the T-Stór record for this article.

13

NOTICE: This is the author's version of a work that was accepted for publication in *Water, Air, & Soil Pollution*. Changes resulting from the publishing process may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Water, Air and Soil Pollution*, 2013;224(9):1693. DOI 10.1007/s11270-013-1693-z. The final publication is available at <http://link.springer.com/article/10.1007/s11270-013-1693-z>.

14

15

This item is made available to you under the Creative Commons Attribution-Non commercial-No Derivatives 3.0 License.

16



17

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

20

21 M.M.R. JAHANGIR^{1,2,3}, P. JOHNSTON², K. ADDY⁴, M.I. KHALIL⁵, P.M. GROFFMAN⁶,
22 K.G. RICHARDS^{1,*}

23

24 ¹Teagasc Environment Research Centre, Johnstown Castle, Co. Wexford, Ireland

25 ²Department of Civil, Structural and Environmental Engineering, Trinity College Dublin,

26 Ireland; ³Department of Soil Science, Bangladesh Agricultural University, Mymensingh-

27 2202; ⁴Dept. of Natural Resources Science, University of Rhode Island, One Greenhouse

28 Road, Kingston, RI 02816, USA; ⁵Environmental Protection Agency, Johnstown Castle

29 Estate, Ireland; ⁶Cary Institute of Ecosystem Studies, P.O. Box AB, Millbrook, NY

30 12545, USA.

31

32

33

34

35

36

37

* Correspondence: Teagasc Environment Research Centre

38

Johnstown Castle

39

Co. Wexford

40

Ireland

41

Tel: +353 (0) 53 9171200

42

E-mail: karl.richards@teagasc.ie

43 **Abstract**

44 Understanding denitrification rates in groundwater ecosystems can help predict where
45 agricultural reactive nitrogen (N) contributes to environmental degradation. *In situ*
46 groundwater denitrification rates were determined in subsoil, at the bedrock-interface and in
47 bedrock at two sites, grassland and arable, using an in situ ‘push-pull’ method with ¹⁵N
48 labelled nitrate (NO₃⁻-N). Measured groundwater denitrification rates ranged from 1.3 to
49 469.5 μg N kg⁻¹d⁻¹. Exceptionally high denitrification rates observed at the bedrock-interface
50 at grassland site (470±152μg N kg⁻¹d⁻¹; SE, standard error) suggest that deep groundwater can
51 serve as substantial hotspots for NO₃⁻-N removal. However, denitrification rates at the other
52 locations were low and may not substantially reduce NO₃⁻-N delivery to surface waters.
53 Denitrification rates were negatively correlated with ambient dissolved oxygen (DO), redox
54 potential (Eh), *k_s* and NO₃⁻ (all p-values p<0.01) and positively correlated with SO₄²⁻
55 (p<0.05). Higher mean N₂O/(N₂O+N₂) ratios at arable (0.28) site than the grassland (0.10)
56 revealed that arable site has higher potential to indirect N₂O emissions. Identification of areas
57 with high and low denitrification and related site parameters can be a tool to manage
58 agricultural N to safeguard the environment.

59 Key words: Denitrification, ¹⁵N-enrichment, ¹⁵N-N₂O, ¹⁵N-N₂, groundwater, N₂O mole
60 fraction

61

62 **1. Introduction**

63 The nitrogen (N) cascade is an increasingly important global issue with multiple impacts
64 on terrestrial, aquatic and atmospheric environments (Galloway et al., 2008). The high rates
65 of N deposition result in N saturation in agricultural land causing high nitrate (NO₃⁻-N)
66 delivery to groundwater which is of concern with result to global environment and human

67 health (Organisation of Economic Co-operation and Development, 2009). In Ireland,
68 groundwater beneath some agricultural systems is contaminated with NO_3^- and this also
69 contributed to the eutrophication of estuarine and near coastal waters (McGarrigle et al.,
70 2010). The OECD (2009) urged Ireland to strengthen measures to achieve “good ecological
71 status” for Irish waters by 2015, paying special attention to eutrophication. The requirement
72 for “good ecological status” for Irish waters is a requirement of the EU Water Framework
73 Directive (WFD; EC, 2002).

74 The biogeochemical process, denitrification, is the principal process which converts the
75 NO_3^- -N to nitrous oxide (N_2O) and dinitrogen (N_2) gas (Rivett et al., 2008). The intermediate
76 product N_2O is a potent greenhouse gas with global warming potential 298 over a 100 year
77 time period. Indirect N_2O emissions resulting from N leaching into associated groundwater
78 are an important but poorly understood component of global N_2O budget (Clough et al.,
79 2007). The quantity of the end product of denitrification process, N_2 , is by far the largest
80 uncertainty of the N cycle at all scales (Galloway et al., 2004). Therefore, narrowing this
81 uncertainty is critical if improvements are to be made in global N_2O and N_2 budgets.
82 Quantification of $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ ratios in groundwater would help refine greenhouse gas
83 inventories and provide insights into the relative contribution of denitrification to
84 environmentally benign N_2 production.

85 As denitrifiers are reported to be ubiquitous in shallow to deep groundwaters (Linne von
86 Berg and Bothe, 1992; Francis et al., 1989) the availability of energy sources and suitability
87 of the hydrogeological environments for denitrifiers need to be investigated. Barrett et al.
88 (2013) quantified denitrification genes in four Irish aquifers (up to 50 m), including the two
89 sites in the current study. They found similar concentrations of denitrification genes across
90 sites and piezometer depth. Therefore optimum hydrogeochemical conditions for microbial

91 denitrification can help biodegradation of NO_3^- -N (ITRC, 2002). Analysis of dissolved N_2O
92 and N_2 in groundwaters from subsoil (5 m), bedrock-interface (12 m) and bedrock (22 m) in
93 Ireland underlined that denitrification can be an important NO_3^- removal pathway across
94 shallow to deep groundwaters (Jahangir et al., 2012a). However, in groundwater
95 denitrification studies it is often unclear if the denitrification products are produced *in situ* or
96 if they have been leached from surface soils (Groffman et al., 1998). Application of *in situ*
97 remediation to any contaminant and site is gaining wide acceptance as viable and economic
98 technology (ITRC, 2002). However, the denitrification process in groundwater is very
99 difficult to measure, and existing methods used to measure denitrification are problematic for
100 a variety of reasons (*e.g.*, high background N_2 , degassing of samples and physical attenuation)
101 (Groffman et al., 2006). The *in situ* NO_3^- push-pull method has been used to determine
102 denitrification in shallow groundwater (<3 m) (Addy et al., 2002; Kellogg et al., 2005). Istok
103 et al. (1997) used the push-pull method for measuring groundwater denitrification in a sand
104 and gravel aquifer at a depth of approximately 10 m. However, in the deep groundwater zones
105 it can be more challenging due to the complex hydrogeological settings *e.g.*, high
106 permeability or preferential flow through fracture in bedrock resulting in high physical
107 attenuation (Buss et al., 2005). In this study, the push-pull method was extended from shallow
108 to deep groundwaters (up to 22 m) to quantify denitrification rates. The objectives of this
109 study were to (a) assess application of the ‘push-pull’ method in deep groundwaters; (b)
110 determine *in situ* denitrification rates in shallow to deep groundwaters; (c) quantify the N_2O
111 mole fractions, $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$; and (d) identify factors controlling the observed spatial trends
112 of denitrification rates.

113

114 **2. Materials and Methods**

115 2.1 Experimental site characteristics

116 The *in situ* NO₃⁻ 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 NO₃-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 NO₃⁻-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

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

143

144 2.4 *In Situ* Push-Pull Pre-test

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

162 ambient level before conducting another pre-test with a shorter incubation period or before
163 conducting the in situ NO_3^- push-pull test.

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

$$168 \quad Mt = \left[\frac{(Vt - Vg)}{\text{Porosity of aquifer}} \right] * Bd \quad (\text{Eqn. 1})$$

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

171

172 2.5 *In Situ* $^{15}\text{N-NO}_3^-$ Push-Pull Experiment

173 *In situ* NO_3^- push-pull tests were conducted in S, I and B with three replications per depth.
174 Twenty liters groundwater was collected in a carboy from each well and stored in a cold room
175 at 4° C for maximum 2 days. To adjust the dissolved oxygen (DO) back to ambient
176 conditions, groundwater solution was bubbled with sulphur hexafluoride (SF_6 ; 98.2%,
177 Cryoservice Ltd., Worcester WR4 9RH, UK) while the DO concentration was monitored
178 using a DO probe (Multi 340i/SET, WTW, Germany). The SF_6 can also serve as a
179 conservative tracer. The dosing solution was prepared with ambient groundwater, 20 mg L^{-1}
180 Br^- as KBr and 20 mg N L^{-1} as isotopically enriched KNO_3 (50 atom% $^{15}\text{N-KNO}_3$; purity
181 99%). The carboy with dosing solution was capped and its headspace was filled with the SF_6
182 gas. The SF_6 headspace was maintained with same pressure while connected to a SF_6 gas
183 cylinder (carried to field) during the injection of the dosing solution. The dosing solution was
184 injected into the respective well over the course of 1- 2-h (depending on the permeability,
185 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, SF₆, 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 SF₆)
199 recoveries were estimated as C/C_0 ; where C was the tracer's concentrations in the pulled
200 groundwater following incubation and C_0 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 (N₂O, N₂ and SF₆) 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 Brunswick Scientific Co., USA) and left for a
209 standing period of 30 min. Headspace samples were then taken for the analysis of SF₆, N₂O

210 and N₂ concentrations and the ¹⁵N enrichment of N₂O and N₂ in 12 ml exetainers (Labco Inc.
211 Wycomb, UK) after injecting additional 12 ml high purity *He*. The N₂O and SF₆ gases were
212 analysed on a gas chromatograph (CP-3800 GC, Varian, Inc. USA/CTC Analytics combi
213 PAL Auto Sampler, Switzerland) equipped with an electron capture detector (ECD) using Ar
214 as a carrier gas. The GC had a Porapak-Q column (80-100 MESH), 3.7 m x 1/8" x 2.0 mm.
215 Concentrations and ¹⁵N enrichment of N₂O and N₂ were determined on a dual-inlet isotope
216 ratio mass spectrometer (Stable Isotope Facility, UC Davis, Davis, CA) as described by
217 Mosier and Schimel (1993).

218

219 2.7 Calculations of Denitrification Rate

220 Dissolved N₂O and N₂ concentrations were calculated using the three highest recovery
221 values within sample replicates (Harrison et al., 2011). The masses of dissolved N₂O–N and
222 N₂ gases (µg) were calculated from the headspace extraction samples using equations and
223 constants provided by Tiedje (1982) and Mosier and Klemetsson (1994). The total mass of
224 N₂O–N or N₂ was then transformed to the mass of ¹⁵N₂O–N or ¹⁵N₂ by multiplying it by the
225 respective ¹⁵N sample enrichment proportion (ratio of pulled atom % of the dissolved N₂O–N
226 and N₂ to pushed NO₃⁻–N atom %, both corrected for ambient atom %). Gas production rates
227 for ¹⁵N₂O–N and ¹⁵N₂–N were expressed as µg N kg⁻¹ soil d⁻¹ as below:

$$228 \quad \text{Rates } \mu\text{g N kg}^{-1} \text{d}^{-1} = \frac{\text{Total mass of } ^{15}\text{N}_2\text{O} - \text{N and } ^{15}\text{N}_2 - \text{N per volume of water pulled}}{\text{Dry mass of soil per volume of water} * \text{incubation period pulled}} \quad (\text{Eqn. 2})$$

229 Mass of aquifer materials was calculated for individual depths at each site. Total
230 denitrification rates were the sum of ¹⁵N₂O–N and ¹⁵N₂ generation rates. All samples used in
231 denitrification calculations contained at least 8 mg L⁻¹ NO₃⁻–N to ensure that calculated
232 denitrification rate estimates were not limited by the amount of NO₃⁻–N available (Schipper
233 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 4°C 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/C_0)

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

262

263 **3. Results**

264 3.1 Groundwater physico-chemical properties

265 Groundwater ambient physico-chemical properties related to denitrification differed
266 among sites (Table 1). Mean NO_3^- -N concentrations were significantly different between sites
267 ($p < 0.001$). Considering the within site differences among various depths, NO_3^- -N
268 concentrations were significantly higher ($p < 0.01$) in S than at the I and B at grassland site but
269 were similar at arable site. Mean NH_4^+ concentrations were low at both sites, with being 0.14
270 and 0.02 mg L^{-1} at grassland and arable sites, respectively. Groundwater pH was near neutral
271 in all depths at grassland but was higher at I compared with B at the arable site. Reduced Fe
272 (Fe II) concentrations were higher at grassland than that at arable site (Table 1). Mean
273 groundwater SO_4^{2-} concentrations were significantly higher at arable site than the grassland
274 ($p < 0.05$) but were similar between depths at each site. Mean S^{2-} concentrations were similar
275 across sites and depths. Mean DO concentrations were significantly lower at the grassland site
276 than at arable site (Table 1). Mean DOC concentrations were significantly higher at grassland
277 ($2.6 \pm 0.8 \text{ mg L}^{-1}$), than at the arable site ($0.9 \pm 0.1 \text{ mg L}^{-1}$) ($p < 0.05$). Interestingly, DOC was
278 similar between depths at each site, whereas DO significantly decreased ($p < 0.05$) with depth
279 at both sites (Table 1). The C/N ratios were significantly higher at grassland than the arable
280 site (data not shown). Irrespective of depths, C/N ratios ranged 1.2 - 20.5 and 0.10 - 0.14 at
281 grassland and arable sites, respectively. Phosphorous (orthophosphate, PO_4^{3-}) concentrations

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

287

288 3.2 Assessment of push-pull method for deep groundwaters

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

300

301 3.3 *In-situ* denitrification rates

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

306 at I (mean = $470 \mu\text{g N kg}^{-1} \text{d}^{-1} \pm 111$); than S (mean = $10.9 \mu\text{g N kg}^{-1} \text{d}^{-1} \pm 3.5$) or B (mean = 9.2
307 $\mu\text{g N kg}^{-1} \text{d}^{-1} \pm 2.8$). Similarly denitrification rates in the three different depths at the arable site
308 were significantly higher ($p < 0.05$) at I ($6.4 \mu\text{g N kg}^{-1} \text{d}^{-1} \pm 1.8$) than S ($3.8 \mu\text{g N kg}^{-1} \text{d}^{-1} \pm 0.7$)
309 or B ($1.4 \mu\text{g N kg}^{-1} \text{d}^{-1} \pm 0.4$) (Figure 3b). Mean denitrification rates were equivalent to a
310 weighted average of 3.92 and $0.09 \text{ mg NO}_3^- \text{-N L}^{-1} \text{d}^{-1}$, 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 $0.3 \text{ mg N L}^{-1} \text{d}^{-1}$
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 N₂O mole fraction

318 The N₂O/(N₂O+ N₂) 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, N₂O/(N₂O+
320 N₂) 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 N₂ 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 ($r = -0.52$, $p < 0.05$), NO₃⁻-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 $330 \text{ ug N kg}^{-1} \text{ d}^{-1}$), Addy et al. (2002) (2.1 to
373 $123.2 \text{ ug N kg}^{-1} \text{ d}^{-1}$) and Harrison et al. (2011) (<0.1 to $193 \text{ ug N kg}^{-1} \text{ d}^{-1}$), 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)

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

378 Our results suggest that while denitrification is not ubiquitous in deep groundwaters, it can
379 serve as substantial hotspots for groundwater N removal before its delivery to surface waters.
380 Higher denitrification rates at the I indicate that denitrification is not limited to shallow
381 groundwater, rather it can occur in deep groundwaters. This notion is in contrast to the
382 assumption of Van Drecht et al. (2003) who developed an empirical model with an
383 assumption that denitrification is zero in deep groundwater. However, underestimation of
384 denitrification rates may also occur because NO_2^- and NO production rates are not included in
385 the calculation (Bollmann and Conrad, 1997; Harrison et al., 2011; Istok et al., 1997).

386 Denitrification rates showed high spatial variability because groundwater hydrogeological
387 properties that control denitrification are heterogeneous. The coefficients of variation of N_2O
388 concentrations between wells within each site, ranged from 55-115 and 47-60% at grassland
389 and arable sites, respectively, and were similar to the coefficients of variation of N_2O
390 production found by other workers, in surface soils e.g., 71-139% (Mathieu et al., 2006), 78-
391 122% (Jahangir et al., 2011), 14-132% (Ishizuka et al., 2005) and in shallow groundwater
392 e.g., 219% (Von der Heide et al., 2008). This variation indicates that denitrification is likely
393 to be an active process, as it is in top soil, of natural NO_3^- reduction in shallow to deep
394 groundwaters. Moreover, high spatial variability of N_2O production is consistent with the high
395 spatial variability of groundwater DO (CV 120%), Eh (CV 219%) and DOC (CV 98%),
396 suggesting that NO_3^- in groundwater is being processed and these properties can be the key
397 indicators of groundwater denitrification. The *in situ* push-pull tests were only conducted
398 during one season because dissolved N_2O and N_2 at the sites were previously observed to be
399 similar throughout the year (Jahangir et al., 2012a).

400 4.3 Variations in N₂O mole fractions

401 Higher N₂O mole fractions at the arable site than that at the grassland might have occurred
402 due to low N₂O reduction rates at this site, because high DO at this site might have reduced
403 N₂O reduction and thus increased its accumulation. Mean N₂O mole fractions in the *in situ*
404 measurements were comparable with those measured in a laboratory incubation of subsoil
405 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
406 0.04 to 0.24 in 120 - 130 cm depths (Jahangir et al., 2012b). The N₂O mole fraction in this
407 study (0.07-0.38) was comparable with Harrison et al. (2011) who measured N₂O/(N₂O+N₂)
408 ratios of 0.02- 0.21 in 0.5 m bgl in alluvial wetlands using the *in situ* push-pull method. Mean
409 N₂O mole fraction, calculated at each site, implies two possibilities: 1) the groundwater could
410 be an important source of atmospheric N₂O when it discharges to surface streams and rivers
411 (Deurer et al., 2008) or diffused upwardly from water table to the atmosphere (Ueda et al.,
412 1993); or 2) N₂O can further be reduced to N₂ (Weymann et al., 2008). Mean mole fractions
413 0.02 at grassland to 0.09 at the arable site from monthly measurements over two years (2009-
414 2010) in these wells (Jahangir et al., 2012a) were lower than that of the measurements by *in*
415 *situ* push-pull test, possibly because N₂O might have been further reduced to N₂ while passing
416 through and from the sediments to the streams due to its longer residence times. However,
417 another possible reason for higher N₂O/(N₂O+N₂) ratios in the *in situ* study than that of the
418 monitoring results of Jahangir et al. (2012a) could be the addition of NO₃⁻-N to groundwater
419 by at least 2 times of the ambient concentration, as high NO₃⁻-N concentration can accelerate
420 N₂O production (Scholefield et al., 1997; Blackmer and Bremner, 1978), inhibit N₂O
421 reduction (Simek and Cooper, 2002) and eventually increase the N₂O mole fraction. The
422 monitoring results suggest that denitrification is more complete, resulting in lower N₂O 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 anaerobicity 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 $13.5 - 4.6 \times 10^3$ and $9.8 - 18.3 \times 10^2$
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 $>10 \text{ mg L}^{-1}$). 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

492 **Acknowledgements**

493 The study was funded by the Department of Agriculture and Food, Ireland through the
494 Research Stimulus Fund Programme (Grant RSF 06383) in collaboration with the Dept. of

495 Civil, Structural and Environmental Engineering, The University of Dublin, Trinity College.
496 The authors sincerely acknowledge the contribution of Mr. John Murphy for in field work.

497

498 **References**

499 Addy, K., Kellogg, D.Q., Gold, A.J., Groffman, P.M., Ferendo, G., & Sawyer, C. (2002). In
500 situ push-pull method to determine groundwater denitrification in riparian zones. *Journal*
501 *of Environmental Quality*, 31, 1017-1024.

502 Aquakem 600A (Konelab 60). (2006). Aquakem Reference Manual. Thermo Electron OY,
503 Clinical Diagnostics, Clinical Chemistry and Automation Systems, Ratiasie 2, Vantaa,
504 Finland.

505 Askew, F.E., & Smith, R.K. (2005). Inorganic non-metallic constituents, 4500-SO₄²⁻;
506 Sulphate; Method 4500-SO₄²⁻ E. Turbimetric method. In: Eaton, et al., (Eds.), Standard
507 Methods for the Examination of Waters and Waste Water, 21st ed. American Public
508 Health Association, NW Washington, DC, pp. 4–188. ISBN 0-87553-047-8 (2001-3710).

509 Barrett, M., Jahangir, M.M.R., Lee, C., Smith, Cindy J., Bhreathnach, N., Collins, G.,
510 Richards, Karl G., & O'Flaherty, V. (2013). Abundance of denitrification genes under
511 different piezometer depths in four Irish agricultural groundwater sites. *Environmental*
512 *Science & Pollution Research*, 20, 6646-6657.

513 Blackmer, A.M., & Bremner, J.M. (1978). Inhibitory effect of nitrate on reduction of nitrous
514 oxide to molecular nitrogen by microorganisms. *Soil Biology and Biochemistry*, 10, 187-
515 191.

516 Böhlke, J.K., Verstraeten, I.M., & Kraemer, T.F. (2007). Effects of surface-water irrigation on
517 sources, fluxes, and residence times of water, nitrate, and uranium in an alluvial aquifer.
518 *Applied Geochemistry*, 22, 152-174.

519 Bollmann, A., & Conrad, R. (1997). Acetylene blockage technique leads to underestimation
520 of denitrification rates in oxic soils due to scavenging of intermediate nitric oxide. *Soil*
521 *Biology and Biochemistry*, 1997; 29: 1067-1077.

522 Bouwer, H., & Rice, R.C. (1976). A slug test for determining hydraulic conductivity of
523 unconfined aquifers with completely or partially penetrating wells. *Water Resources*
524 *Research*, 12, 423-428.

525 Buss, S.R., Rivett, A.R., Morgan, P., & Bemment, C.D. (2005). Attenuation of nitrate in the
526 subsurface environment. *Environment Agency Report*, EPA, UK.

527 Clough, T.J., Addy, K., Kellogg, D.Q., Nowicki, B.L., Gold, A.J., & Groffman, P.M. (2007).
528 Dynamics of nitrous oxide in groundwater at the aquatic-terrestrial interface. *Global*
529 *Change Biology*, 13, 1528-1537.

530 Davidson, E.A., & Firestone, R.K. (1988). Measurement of nitrous oxide dissolved in soil
531 solution. *Soil Science Society of America Journal*, 52, 1201-1203.

532 Deurer, M., Von der Heide, C., Bottcher, J., Duijnsveld, W.H.M., Weymann, D., & Well, R.
533 (2008). The dynamic of N₂O near the groundwater table and the transfer of N₂O into the
534 unsaturated zone: A case study from a sandy aquifer in Germany. *Cataena*, 72, 362-373.

535 EC (2002). Council Directive (2000/60/EEC) of the 23rd October 2000 on establishing a
536 framework for Community action in the field of water policy. Official Journal of the
537 European Communities L327/1, Brussels, Belgium.

538 Fenton, O., Richards, K.G., Khalil, M.I., & Healy, M.G. (2009). Factors affecting nitrate in
539 shallow groundwater under a beef farm in South Eastern Ireland. *Journal of*
540 *Environmental Management*, 90 (10), 3135–3146.

541 Fenton, O., Schulte, R.P.O., Jordan, P., Lalor, S.T.J., & Richards, K.G. (2011). Time lag: a
542 methodology for the estimation of vertical and horizontal travel and flushing timescales to
543 nitrate threshold concentrations in Irish aquifers. *Environmental Science and Policy*,
544 14(4), 419-431.

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

548 Francis, A.J., Slater, J.M., & Dodge, C.J. (1998). Denitrification in deep sub-surface
549 sediments. *Geomicrobiology Journal*, 7, 103-106.

550 Freeze, R.A., & Cherry, J.A. (1979). *Ground Water*. Prentice Hall, Englewood Cliffs, New
551 Jersey.

552 Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z.C., Freney, J.R.,
553 Martinelli, L.A., Seitzinger, S.P., & Sutton, M.A. (2008). Transformation of the N cycle:
554 recent trends, questions and potential solutions. *Science* 320, 889-892.

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

559 GenStat. Version 13.1, VSN International Ltd. 2011; UK.

560 Groffman, P.M., Gold, A.J., & Jacinthe, P.A. (1998). Nitrous oxide production in riparian
561 zones and groundwater. *Nutrient Cycling Agroecosystems*, 52, 179-186.

562 Groffman, P.M., Altabet, M.A., Böhlke, J.K., Butterbach-Bahl, K., David, M.B., Firestone,
563 M.K., Giblin, A.E., Kana, T.M., Nielsen, L.P., & Voytek, M.A. (2006). Methods for
564 measuring denitrification: Diverse approaches to a difficult problem. *Ecological*
565 *Applications*, 16, 2091–2122.

566 Harrison, M.D., Groffman, P.M., Mayer, P.M., Kaushal, S.S., & Newcomer, T.A. (2011).
567 Denitrification in Alluvial Wetlands in an Urban Landscape. *Journal of Environmental*
568 *Quality*, 40, 634-646.

569 Ishizuka, S., Iswandi, A., Nakajima, Y., Yonemura, S., Sudo, S., Tsuruta, H., & Muriyarso, D.
570 (2005). Spatial patterns of greenhouse gas emission in a tropical rainforest in Indonesia.
571 *Nutrient Cycling in Agroecosystems*, 71, 55-62.

572 Interstate Technology and Regulatory Council (2002). A systematic approach to in situ
573 bioremediation in groundwater. Technical/Regulatory Guidelines, August 2002, USA.

574 Istok, J.D., Humphrey, M.D., Schroth, M.H., Hyman, M.R., & O'Reilly, K.T. (1997). Single-
575 Well, “Push-Pull” Test for In Situ Determination of Microbial Activities. *Ground Water*
576 35, 619–631.

577 Jahangir, M.M.R., Roobroek, D., Van Cleemput, O., & Boeckx, P. (2011). Spatial variability
578 and biophysicochemical controls on N₂O emissions from differently tilled arable soils.
579 *Biology and Fertility of Soils*, 47(7), 753-766.

580 Jahangir, M.M.R., Khalil, M.I, Johnston, P.M., Cardenas, L., Butler, M., Hatch, D., Barrett,
581 M.M., O’Flaherty, V., & Richards, K.G. (2012b). Denitrification potential in subsoils: a

582 mechanism to reduce nitrate leaching to groundwater. *Agriculture Ecosystems &*
583 *Environment*, 147, 13-23.

584 Jahangir, M.M.R., Johnston, P.M., Barrett, M., Khalil, M.I., Fenton, O., Boeckx, P.,
585 Groffman, P., & Richards, K.G. (2012a). Denitrification and indirect N₂O emissions from
586 groundwater: Hydrologic and biogeochemical influences. *Journal of Contaminant*
587 *Hydrology*, 152, 70-81.

588 Kana, T.M., Darkangelo, C., Hunt, M.D., Oldham, J.B., Bennett, G.E., & Cornwell, J.C.
589 (1994). Membrane inlet mass spectrometer for rapid high precision determination N₂, O₂
590 and Ar in environmental water samples. *Analytical Chemistry*, 66, 4166-4170.

591 Kellogg, D.Q., Gold, A.J., Groffman, P.M., Addy, K., Stolt, M.H., & Blazewski, G. (2005).
592 In situ groundwater denitrification in stratified, permeable soils underlying riparian
593 wetlands. *Journal of Environmental Quality*, 34, 524-533.

594 Kollé, W., Stöbel, O., & Bottcher, J. (1985). Formation of sulphate by microbial
595 denitrification in a reducing aquifer. *Water Supply*, 3, 35-40.

596 Konrad, C. (2007). Methoden zur Bestimmung des Umsatzes von Stickstoff, dargestellt für
597 drei pleistozane Grundwasserleiter Nord-deutschlands, PhD thesis, Dresden University
598 Of Technology, Germany, p. 157.

599 Lemon, E. (1981). Nitrous oxide in freshwaters of the Great Lakes basins. *Limnology and*
600 *Oceanography*, 26, 867-879.

601 Linne von Berg, K.H., & Bothe, H. (1992). The distribution of denitrifying bacteria in soil
602 monitored by DNA-probing. *FEMS Microbiology Ecology*, 86, 331-340.

- 603 Mathieu, O., Leveque, J., Heault, C., Milloux, M.J., Bizouard, F., & Andreux, F. (2006).
604 Emissions and spatial variability of N₂O, N₂ and nitrous oxide mole fraction at the field
605 scale, revealed with ¹⁵N isotopic techniques. *Soil Biology and Biochemistry*, 38, 941-951.
- 606 McGarrigle, M., Lucey, J., & Ó’Cinnéde, M. (2010). Water Quality in Ireland 2007-2009.
607 Synthesis Report, Environmental protection Agency, Wexford, Ireland.
- 608 Misstear, B.D.R., Brown, L., & Johnston, P.M. (2009). Estimation of groundwater recharge in
609 a major sand and gravel aquifer in Ireland using multiple approaches. *Hydrogeology*
610 *Journal*, 17, 693-706.
- 611 Mosier, A.R., & Schimel, D.S. (1993). Nitrification and Denitrification. In: Knowles, R., and
612 T.H. Blackburn. (Ed.). Nitrogen isotope techniques. Academic Press, 1993; Orlando, FL.
613 pp. 181-208.
- 614 Mosier, A.R., & Klemetsson, L. (1994). Measuring denitrification in the field. In: Weaver et
615 al. (Ed.). Methods of Soil Analysis. Part 2, SSSA Book Ser 5, SSSA, 1994; Madison,
616 USA. pp. 1047-1065.
- 617 Nelson, W.M., Gold, A.J., & Groffman, P.M. (1995). Spatial and temporal variation in
618 groundwater nitrate removal in a riparian forest. *Journal of Environmental Quality*, 24,
619 691-699.
- 620 Organisation of Economic Co-operation and Development. (2009). OECD Environmental
621 Performance Reviews of Ireland, Conclusions and Recommendations. 4 November
622 2009, p. 2-18.
- 623 Ott, R.L. (1993). An introduction to statistical methods and data analysis. 4th Ed. Duxbury
624 Press, Belmont, CA.

- 625 Premrov A., Coxon C.E., Hackett R., Kirwan L., & Richards K.G. (2012). Effects of over-
626 winter green cover on groundwater nitrate and dissolved organic carbon concentrations
627 beneath tillage land, *Science of the Total Environment*, 438, 144–153.
- 628 Richards, K., Coxon, C.E., & Ryan, M. (2005). Unsaturated zone travel time to groundwater
629 on a vulnerable site. *Irish Geography*, 38, 57-71.
- 630 Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.S.N., & Bemment, C.D. (2008). Nitrate
631 attenuation in groundwater: A review of biogeochemical controlling processes. *Water*
632 *Research*, 42, 4215-4232.
- 633 Robertson, L.A., Dalsgaard, T., Revsbech, N.P., & Kuenen, J. (1995). Confirmation of
634 ‘aerobic denitrification’ in batch cultures, using gas chromatography and ¹⁵N mass
635 spectrometry. *FEMS Microbiol Ecology*, 18, 113–120.
- 636 Schipper, L., & Vojvodic-Vukovic, M. (1998). Nitrate removal from groundwater using a
637 denitrification wall amended with sawdust: Field trial. *Journal of Environmental Quality*,
638 27, 664-668.
- 639 Scholefield, D., Hawkins, J.M.B., & Jackson, S.M. (1997). Use of flowing helium atmosphere
640 incubation technique to measure the effects of denitrification controls applied to intact
641 cores of a clay soil. *Soil Biology and Biochemistry*, 29, 1337-1344.
- 642 Seitzinger, S., Harrison, J.A., Böhlke, J.K., Bouwman, A.F., Lowrance, R., Peterson, B.,
643 Tobias, C., & Van Drecht, G. (2006). Denitrification across landscape and waterscape: A
644 synthesis. *Ecological Application*, 16, 2064–2090.
- 645 Simek, M., Cooper, J.E. (2002). The influence of soil pH on denitrification: Process towards
646 the understanding of this interaction over the last 50 years. *European Journal of Soil*
647 *Science*, 53, 345-354.

- 648 Simmons, R.C., Gold, A.J., & Groffman, P.M. (1992). Nitrate dynamics in riparian forests:
649 Groundwater studies. *Journal of Environmental Quality*, 21, 656-665.
- 650 Starr, J.L., Sadeghi, A.M., Parkin, T.B., & Meisinger, J.J. (1996). (1996). A tracer test to
651 determine the fate of nitrate in shallow groundwater. *Journal of Environmental Quality*,
652 25, 917-923.
- 653 Tiedje, J.M. (1982). Denitrification. p. 1011-1025. In: Page et al. (Ed.). Methods of soil
654 analysis. Part 2, 2nd Ed. Agron. Monogr. ASA and SSSA, Madison, WI.
- 655 Ueda, S., Ogura, N., Yoshinary, T. (1993). Accumulation of nitrous oxide in aerobic
656 groundwaters. *Water Research*, 27, 1787-1792.
- 657 Van Drecht, G., Bouwman, A.F., Knoop, J.M., Beusen, A.H.W., Meinardi, C.R. (2003).
658 Global modelling of the fate of nitrogen from point and non-point sources in soils,
659 groundwater, and surface water. *Global Biogeochemical Cycles*, 17(4), 1115.
660 doi:10.1029/2003GB002060
- 661 Vogel, J.C., Talma, A.S., & Heaton, T.H.E. (1981). Gaseous nitrogen as evidence for
662 denitrification in groundwater. *Journal of Hydrology*, 50(1-3), 191-200.
- 663 Von der Heide, C., Böttcher, J., Deurer, M., Weymann, D., Well, R., & Duijnisveld, W.H.M.
664 (2008). Spatial variability of N₂O concentrations and of denitrification-related factors in
665 the surficial groundwater of a catchment in Northern Germany. *Journal of Hydrology*,
666 360, 230-241.
- 667 Weymann, D, Geistlinger H, Well R, Von der Heide C, & Flessa H. (2010). Kinetics of N₂O
668 production and reduction in a nitrate-contaminated aquifer inferred from laboratory
669 incubation experiments. *Biogeosciences*, 7: 1953-1972.

670 Weymann, D., Well, R., Flessa, H., von der Heide, C., Deurer, M., Meyer, K., Konrad, C., &
671 Walther, W. (2008). Groundwater N₂O emission factors of nitrate-contaminated aquifers
672 as derived from denitrification progress and N₂O accumulation. *Biogeosciences*
673 *Discussion*, 5, 1215-1226.

674

675

676

677

678

679

680

681

682 *Table 1* Ambient hydrologic and hydrochemical properties; values are means \pm SE, n=3

Depth	NO ₃ ⁻ -N	DOC‡	Fe (II)mg L ⁻¹	SO ₄ ²⁻	S ²⁻	DO‡	pH	Eh (m V)	GWT (m, bgl)	k _s (m d ⁻¹)
Grassland										
Subsoil 5 m bgl	4.7 \pm 1.6a	1.0 \pm 0.1a	12 \pm 4a	20.0 \pm 1.6a	0.26 \pm 0.04a	1.9 \pm 0.1a	6.9 \pm 0.1a	94 \pm 28a	1.8 \pm 0.1a	0.008 \pm 0.002a
Interface 12 m bgl	2.0 \pm 1.8b	3.5 \pm 2.3a	48 \pm 27b	19.2 \pm 1.6a	0.21 \pm 0.06a	1.3 \pm 0.4b	6.8 \pm 0.1a	25 \pm 62b	2.9 \pm 0.9b	0.024 \pm 0.004a
Bedrock 22 m bgl	2.9 \pm 1.3b	3.4 \pm 2.7a	14 \pm 13a	16.4 \pm 1.6a	0.24 \pm 0.04a	1.6 \pm 0.1b	6.8 \pm 0.1a	47 \pm 43b	3.4 \pm 1.0b	0.030 \pm 0.005a
Arable land										
Subsoil 5 m bgl	12.8 \pm 2.6a	1.1 \pm 0.2a	4.4 \pm 1.1a	27.2 \pm 1.0a	0.17 \pm 0.01a	9.5 \pm 1.4a	7.8 \pm 1.3b	178 \pm 60a	4.2 \pm 0.2a	0.033 \pm 0.006a
Interface 12 m bgl	10.4 \pm 0.3a	0.8 \pm 0.2a	4.8 \pm 0.7a	23.3 \pm 1.1a	0.24 \pm 0.08a	6.2 \pm 1.6b	8.9 \pm 1.2a	163 \pm 50a	4.6 \pm 0.1a	0.053 \pm 0.003a
Bedrock 22 m bgl	12.6 \pm 2.5a	0.7 \pm 0.2a	2.7 \pm 1.0a	27.3 \pm 0.7a	0.18 \pm 0.05a	4.1 \pm 1.4b	7.5 \pm 0.1b	107 \pm 39b	5.1 \pm 0.1a	0.123 \pm 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)
685
686

687

688

689

690

691

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
697 Park (OP) with average water table and Ks values. Wells installation depths, geochemical
698 properties, details of water table depths and Ks values were summarised in Table 1

699 *Figure 3* Mean denitrification rates (N_2O+N_2) in (a), three different depths of groundwater
700 at grassland (n=3) and (b), at arable land (n=3)

701 *Figure 4* Mean N_2O mole fraction, $N_2O/(N_2O+N_2)$ in (a), three different depths of
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

705

706

707

708

709

710

711

712

713

714

715