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10 Exploring the relationship between groundwater geochemical factors and
11 denitrification potentials on a dairy farm in south east Ireland.
12

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24

25 **ABSTRACT**

26 Nitrate (NO₃⁻) loss from agriculture to shallow groundwater and transferral to
27 sensitive aquatic ecosystems is of global concern. Denitrifying bioreactor technology,
28 where a solid carbon (C) reactive media intercepts contaminated groundwater, has
29 been successfully used to convert NO₃⁻ to di-nitrogen (N₂) gas. One of the challenges
30 of groundwater remediation research is how to track denitrification potential spatially
31 and temporally within reactive media and subsoil. First, using δ¹⁵N/δ¹⁸O isotopes,
32 eight wells were divided into indicative transformational processes of ‘nitrification’ or
33 ‘denitrification’ wells. Then, using N₂/argon (Ar) ratios these wells were divided into
34 ‘low denitrification potential’ or high denitrification potential’ categories. Secondly,
35 using falling head tests, the saturated hydraulic conductivity (K_{sat}) in each well was
36 estimated, creating two groups of ‘slow’ (0.06 m day⁻¹) and ‘fast’ (0.13 m day⁻¹)
37 wells, respectively. Thirdly, two ‘low denitrification potential’ wells (one fast and one
38 slow) with high NO₃⁻ concentration were amended with woodchip to enhance
39 denitrification. Water samples were retrieved from all wells using a low flow syringe

40 to avoid de-gassing and analysed for N₂/Ar ratio using Membrane Inlet Mass
41 Spectrometry. Results showed that there was good agreement between isotope and
42 chemical (N₂/Ar ratio and dissolved organic C (DOC)) and physio-chemical
43 (dissolved oxygen, temperature, conductivity and pH) parameters. To explain the
44 spatial and temporal distribution of NO₃⁻ and other parameters on site, the
45 development of predictive models using the available datasets for this field site was
46 examined for NO₃⁻, Cl⁻, N₂/Ar and DOC. Initial statistical analysis was directed
47 towards the testing of the effect of woodchip amendment. The analysis was
48 formulated as a repeated measures analysis of the factorial structure for treatment and
49 time. Nitrate concentrations were related to K_{sat} and water level ($p < 0.0001$ and $p = 0.02$
50 respectively), but did not respond to woodchip addition ($p = 0.09$). This non-
51 destructive technique allows elucidation of denitrification potential over time and
52 could be used in denitrifying bioreactor technology to assess denitrification hotspots
53 in reactive media, while developing a NO₃⁻ spatial and temporal predictive model for
54 bioreactor site specific conditions.

55

56 **Keywords:** bioreactor; denitrification; isotope; MIMS; nitrate; groundwater; Water
57 Framework Directive.

58

59 **1. Introduction**

60 Excess reactive nitrogen (N), such as nitrate (NO₃⁻) and ammonia (NH₃), which
61 contribute to eutrophication of aquatic/terrestrial ecosystems and the potent
62 greenhouse gas, nitrous oxide (N₂O), are of global concern (Stark and Richards,
63 2008). The loss of N from agricultural land to a waterbody is a serious concern in
64 river basin management in many countries (Kurz et al., 2005).

65

66 Subsoil denitrifying bioreactors are used to remediate shallow groundwater NO_3^-
67 pollution from point sources, and comprise organic carbon (C) media placed in the
68 flow path of NO_3^- contaminated groundwater. The C in the media acts as an energy
69 source to support denitrification and the conversion of NO_3^- into di-nitrogen (N_2) and
70 N_2O gases (Carmichael, 1994), or through a dissimilatory nitrate reduction to
71 ammonium (NH_4) (DNRA) pathway. The application of such reactive media has been
72 applied in permeable reactive barriers (denitrification walls) (Schipper et al., 2004,
73 2005; Robertson and Merkley, 2009), drain pipe installations (Jaynes et al., 2004), soil
74 reactive layers, effluent beds and wetlands (van Driel et al., 2006; Robertson and
75 Schiff, 2008; Robertson and Merkley, 2009; Hien et al., 2010).

76

77 A knowledge gap in denitrifying bioreactor research is the methodological constraints
78 that prevent the direct measurement of denitrification within a bioreactor, the
79 identification of 'denitrification hotspots', and denitrification potential changes over
80 time. To overcome such limitations, studies have calculated NO_3^- removal rates in
81 laboratory experiments with reactive media of different ages (Robertson, 2010), or by
82 quantifying the performance of bioreactors over time (Schipper and Vojvodic-
83 Vukovic, 2001). As monitoring of NO_3^- removal in bioreactors is performed using
84 narrow diameter wells, a methodology to determine denitrification potential that uses
85 this existing well network may be beneficial.

86

87 Whereas natural abundance stable isotopic techniques identify possible NO_3^- sources
88 and transformational processes in a monitoring well, N_2 /argon (Ar) ratios point to
89 denitrification potential in the same well. As NO_3^- sources have distinct nitrogen (δ

90 ^{15}N) and oxygen ($\delta^{18}\text{O}$) isotopic compositions, N sources, such as chemical fertilizer,
91 manure, and atmospheric deposition (Kendall, 1998); and processes such as
92 denitrification (Aravena and Robertson, 1998; Fukada et al., 2004) and nitrification
93 (Kendall, 1998) can be ascertained in a groundwater monitoring well. Groffman et al.
94 (2006) reviewed methodologies to determine the spatial and temporal extent of
95 denitrification potential and suggested that direct N_2 and N_2/Ar measurements in
96 aquatic and marine systems using Membrane Inlet Mass Spectroscopy (Kana et al.,
97 1998) should be investigated further. The novelty of this high through-put technique is
98 that it requires a small sample of groundwater (< 10 ml); it eliminates a manned
99 degassing step, and has an error of $< 0.5\%$ for N_2 and Ar concentrations across a wide
100 range of temperature equilibriums.

101

102 The objectives of the current study, based on a dairy farm with shallow groundwater
103 NO_3^- in south east Ireland, were to:

104

- 105 1. Identify different NO_3^- sources and transformational processes within a
106 monitoring network using natural abundance
- 107 2. Identify high/low denitrification potential wells using N_2/Ar ratios
- 108 3. Investigate N_2/Ar ratios over time in: low/high denitrification wells and C
109 enhanced low denitrification wells with different saturated hydraulic
110 conductivities, K_{sat} .
- 111 4. Determine factors of importance in the development of predictive spatial and
112 temporal distribution models for groundwater geochemical factors (nitrate and
113 chloride) and denitrification potentials (N_2/Ar , dissolved organic C (DOC),
114 total organic C (TOC)) using K_{sat} and other variables.

115

116 **2. Methods**

117 *2.1 Study site description*

118 The 20 ha study site was situated on a dairy farm 60.8 ha in size, located at Johnstown
119 Castle, Wexford, south east Ireland (latitude 52° 12 N, longitude 6° 30 W) (Fig 1).

120 This location has a temperate maritime climate, with rainfall ranging from 1000 to
121 1400 mm and a mean annual temperature of 9.6 °C. The site comprises mature,
122 medium permeability till overburden (K_{sat} , $5 \times 10^{-8} \text{ m s}^{-1}$ to $5 \times 10^{-4} \text{ m s}^{-1}$), which is
123 heterogeneous and morainic in nature. Vertical unsaturated travel times (months to
124 years) on site varies for each well based on unsaturated zone thickness, effective
125 rainfall (600 mm) and effective porosity (η_e) (from 5 to 30%) (Fenton et al., 2010).

126 The soil is underlain by an unproductive, low conductivity pre-Cambrian greywacke,
127 schist and massive schistose quartzites that have been subjected to low-grade
128 metamorphism. Depth to bedrock on site is approximately 12 m. The study site
129 consists of eight wells (25 mm LDPE casing; Van Walt Ltd, Surrey, U.K.) installed to
130 shallow groundwater (mean drilling depth was 6.3 m), using rotary drilling (60 mm in
131 diameter) – using a Giddings soil excavation rig to several metres below water strike.

132

133 *2.2 Isotopic signatures*

134 Using a subset of isotopic results from Baily et al. (submitted), the occurrence of
135 denitrification in April (spring), August (summer) and December (autumn) of 2008 in
136 the studied wells was determined. Baily et al. (submitted) showed that the spatial
137 pattern of NO_3^- in shallow groundwater differs, but, as the mild and moist climate
138 present on this site allows biological processes to continue all year round, the
139 temporal pattern is relatively constant. The temporal nature of the site allowed the

140 current study to be carried out at any time of the year. A summary of results from the
141 Baily et al. (submitted) study is presented in Table 1.

142

143 *2.3 K_{sat} , watertable height and effective rainfall*

144 The K_{sat} of each well was calculated using the Bouwer and Rice slug injection test
145 method (Bouwer and Rice, 1976). A mini electronic diver (Van Walt, U.K.), set at a
146 0.5 s resolution, collected drawdown data. The linear part of the response curve was
147 tested, after which the influence of the filter pack had dissipated. Whereas isotopic
148 results identified the shallow groundwater NO_3 source and differentiated wells into
149 ‘nitrification’ and ‘denitrification’ transformational categories, K_{sat} divided such wells
150 further into high and low permeability within medium permeability tills. Wells were
151 dipped for watertable depth each day to ensure the screened intervals of the wells
152 were saturated at all times. Daily meteorological data was collected from the
153 Johnstown Castle Weather Station, which is located on the farm. To estimate daily
154 effective rainfall, temperature, total rainfall, wind speed, solar radiation and humidity
155 were inputted into a hybrid model designed for grassland in Ireland (Schulte et al.,
156 2005). This was to investigate recharge to shallow groundwater during the experiment
157 and to elucidate the effects of dilution on NO_3^- concentration. The major mechanism
158 of NO_3^- remediation is dilution and denitrification. If denitrification is dominant,
159 isotope composition is heavier in times of high recharge.

160

161 *2.4. Water samples*

162 Use of peristaltic or low flow pumps can be limiting in bioreactor studies due to the
163 inner diameter of wells. Such equipment can cause degassing of water samples and
164 make them unsuitable for use in groundwater investigations. To overcome de-gassing,

165 in each well, gas impermeable tubing, with an inner diameter 5 mm, was installed to
166 the centre of the screen interval. At surface level, a three-way stop cock and 50 ml
167 syringe were attached. To elucidate potential denitrification in the screened interval of
168 each well based on dissolved N₂ and the N₂/Ar ratio (Kana et al., 1998; An et al.,
169 2001), three water samples at selected sample dates were taken (24th -25th and 27th-
170 28th August, 1st, 4th, 10th, 17th, 24th, 30th September, 8th, 16th, 28th October, 5th
171 November, 2009). Water samples were transferred from the syringe to a 12 ml
172 Exetainer® (Labco Ltd, U.K.) filled from the base of each container, overfilled, and
173 then sealed to avoid any air entrapment with a butyl rubber septum. Samples were
174 then placed upside down under water (below the average groundwater temperature of
175 12°C) in an ice box, transported to the laboratory, and kept in a cold room at 4°C prior
176 to analysis. Dissolved N₂, O₂ and Ar were analyzed using a Membrane Inlet Mass
177 Spectrometry (MIMS) at the temperature measured (11°C) during groundwater
178 sampling (Kana et al. 1998).

179

180 Calculation of excess N₂ was after Weymann et al. (2008), using 15°C water bath
181 temperature, pressure 755 mm HG, based on elevation of site above sea level and a
182 headspace temperature of 15°. In addition, Reaction Progress (RP), representing the
183 extent of NO₃⁻ elimination, was calculated after Böhlke et al. (2002) by dividing the
184 denitrification product, N₂ (considering N₂O production was negligible as reported by
185 Khalil and Richards (2010)), by the initial concentration of NO₃⁻ and excess N₂.

186

187 Nutrient concentrations of water samples were determined using a Thermo Konelab
188 20 analyser (Technical Laboratory Services, Ontario, Canada) for NO₂-N, total
189 oxidised N (TON-N), ammonium-N (NH₄-N), chloride (Cl⁻), total dissolved nitrogen

190 (TDN), total phosphorus (TP) and calcium (Ca^{2+}). $\text{NO}_3\text{-N}$ was determined by
191 subtracting $\text{NO}_2\text{-N}$ from TON. Water samples were collected in polyethylene screw
192 top bottles and filtered through a $0.45\ \mu\text{m}$ filter membrane. In addition, other
193 parameters were taken to investigate if denitrification is a viable pathway for NO_3^-
194 reduction. pH, conductivity (cond, $\mu\text{S cm}^{-1}$) and temperature (temp, $^\circ\text{C}$) were
195 measured in the field using a multi-parameter Troll 9500 probe (In-situ, Colorado,
196 U.S.A.) with a flow through cell. Dissolved organic carbon and TOC were also
197 measured using a TOC-V Series (Shimadzu, Kyoto, Japan). The metal content of the
198 water samples (copper (Cu), iron (Fe), magnesium (Mg), potassium (K) and zinc
199 (Zn)) were determined by aqua regia digestion using a Gerhard Block digestion
200 system (Cottenie and Kiekens, 1984) and analysed using an ICP VISTA-MPX
201 (Varian, California, U.S.A.).

202

203 *2.5 Solid carbon enhancement*

204 Washed, untreated woodchip (WC) (10 g, 1-2 mm in length) was packed loosely into
205 a filter sock (Eijelkamp, the Netherlands) approximately 0.2 m in length and 0.02 m
206 thick. This was cable tied and installed in the screened interval of two wells (L1 (WC)
207 and L3 (WC)) with high NO_3^- concentration, a 'low denitrification potential' signal
208 from N_2/Ar analysis and with low (L1 (WC)) and high (L3 (WC)) K_{sat} with moderate
209 permeability tills.

210

211 *2.6 Statistical analysis*

212 To explain the spatial and temporal distribution of NO_3^- and other parameters on site,
213 the development of predictive models using the available datasets for this field site
214 was examined for NO_3^- , Cl^- , N_2/Ar and DOC. As a first step, statistical analysis was

215 directed towards the testing of the effect of woodchip enhancement in Wells L1 (WC)
216 and L3 (WC). The analysis was formulated as a repeated measures analysis of the
217 factorial structure for treatment and time. Statistical analysis was undertaken with
218 group variables: WC (L1 (WC) and L2 (WC)) and all other wells were set up as a
219 fixed, treatment effect in the analysis using SAS V.9 (2003) software. A factorial
220 model with days and treatment (WC or other wells) and their interaction was
221 developed. As readings within each well were correlated and as the time between
222 sampling events varied, a spatial type covariance structure was fitted across days (14
223 days in total, between 24th August 2008 and 5th November 2009). Mixed models were
224 used to account for the repeated measures in each well and for heterogeneous variance
225 within treatments. As previous work (Fenton et al., 2009) correlated K_{sat} as an
226 important parameter to explain denitrification, testing it for inclusion as a covariate in
227 any analysis was essential for an unbiased test of the treatment effect. Other
228 covariates were also tested.

229

230 A number of covariates were available (K_{sat} , O_2 and depth to watertable) and these
231 were tested for inclusion in the model to remove possible bias and for their potential
232 role in a predictive model. As the number of K_{sat} values varied spatially but not
233 temporally, and the relationship between variables examined and K_{sat} was statistically
234 significant, apparent non-linearity in the examined relationships was modelled by a
235 nonlinear regression fitted with mixed model equations using Proc Nlmixed (SAS,
236 2003). Residual checks were made to ensure the approach used, but did not violate the
237 assumptions of the analysis. Transformation was used as required for the variable
238 being analysed.

239 **3. Results**

240 *3.1 K_{sat} , watertable depth and effective rainfall*

241 The K_{sat} on site for each well is presented in Table 2. Combining such results with
242 isotopic data from Table 1 allowed the wells to be paired, e.g. Wells L1 (WC) and L2.
243 Both wells had the same ‘low denitrification potential’ signal and both had a low K_{sat}
244 of 0.06 m day⁻¹. Similarly, Wells L3 (WC) and L4 had a ‘low denitrification potential’
245 signal, but had a high K_{sat} of 0.13 m day⁻¹. Wells H1-H3 were identified by isotope
246 analysis as ‘high denitrification potential’ wells and had a very low K_{sat} , allowing
247 enough time for denitrification to occur. The depth to watertable during the
248 experiment was consistently above the screened interval depth, ensuring the woodchip
249 was saturated throughout the experiment (Table 2).

250

251 Using the K_{sat} of each well, a uniform hydraulic gradient of 1% and effective porosity
252 of 5%, effective velocity ranged from 0.001 to 0.02 m day⁻¹. This is the equivalent of
253 0.05 to 0.78 years required to travel 5 m down gradient of the well network. The
254 experiment lasted for 0.24 years and water movement in each well was very slow
255 during this period.

256

257 *3.2 Water samples*

258 The N₂/Ar signal of ‘low denitrification potential’ was changed in well L1 (WC) by
259 woodchip addition. This was associated with an unchanged mean Cl⁻ concentration
260 and a pronounced decrease in NO₃⁻ concentration during the experiment, indicating
261 denitrification. Chloride release from the woodchip was also expected, showing
262 interaction with the contaminated water and the well. Despite the N₂/Ar signal of ‘low
263 denitrification potential’ in wells L2 – L5, low mean Cl⁻ concentration with
264 pronounced decreases in NO₃⁻ concentration was observed. This indicated possible

265 dilution in 'low denitrification potential' wells L2-L5 (Table 2). The isotope data from
266 April to August, the 'low denitrification potential' well L1 (WC) and all 'high
267 denitrification potential' wells had composition values higher in August than in April.
268 For 'low denitrification potential' wells L2, L4 and L5, this decreased, with no change
269 in L3 (WC). Therefore, some dilution occurred in Wells L2, L4 and L5. Recharge
270 occurred from day 5 to 24. N_2/Ar ratios, NO_3^- and Cl^- concentration throughout the
271 experiment for all wells are presented in Fig 2. Looking at isotopic compositions from
272 Table 1, values tended to be higher in the N_2/Ar results for 'low denitrification
273 potential' well L3 (WC), with high K_{sat} . As expected from the isotopic results, 'high
274 denitrification potential' wells (H1-H3) had low mean Cl^- and NO_3^- concentrations,
275 confirming natural abundance results i.e. transformational process signal of
276 denitrification (Table 2).

277

278 A denitrification potential background N_2/Ar ratio based on minimum dissolution of
279 entrapped air in the groundwater sample was found to be 37.68 (Weymann et al.,
280 2008). Denitrification occurs above this value in the groundwater. Between 'low
281 denitrification potential' wells L1 (WC) and L2, well L1 (WC) after C enhancement
282 consistently demonstrated higher denitrification potential. Again, between 'low
283 denitrification potential' wells L3 (WC) and L4, well L3 (WC) consistently
284 demonstrated higher denitrification potential. Both groups had different K_{sat} , but K_{sat}
285 was the same within each group. Among the wells with low K_{sat} , well L1 (WC)
286 showed greatest denitrification potential. In 'high denitrification potential' wells, the
287 N_2/Ar ratios were consistently above 40.

288

289 The extent of denitrification in a water sample is the excess N_2 , accounting for the
290 solubility and excess air. Conforming to results derived from isotopic signatures, the
291 average NO_3^- -N, represented as excess N_2 , was found to be higher in 'high
292 denitrification potential' wells compared with 'low denitrification potential' wells.
293 However, the mean amount of NO_3^- removal due to denitrification in all wells was
294 small (Table 3). For C enhanced wells and 'high denitrification potential' wells, this
295 mean NO_3^- removal due to denitrification was approximately $1 \text{ mg } NO_3\text{-N L}^{-1}$. Solid C
296 emplacement in 'low denitrification potential' wells L1 (WC) and L3 (WC) only
297 resulted in a small increase in the denitrification potential. Denitrification progress
298 reduces the amount of NO_3^- , but calculated RP mostly produced similar results for the
299 full extent of NO_3^- elimination (Table 3). In 'low denitrification potential' wells, the
300 RP varied from 0 to 0.06 and was highest in the wells containing WC. It increased the
301 most in 'high denitrification potential' wells, which enhanced reduction of NO_3^- from
302 18 (H2) to 91% (H3).

303

304 In Fig. 3a, the 'low denitrification potential' wells reached steady state early in the
305 experiment, with no changes after approximately 10 days. The 'low denitrification
306 potential' well L1 (WC), however, continued to increase throughout the experiment.
307 For the 'high denitrification potential' wells in Fig. 2, well H2 showed a constant
308 reduction of NO_3^- throughout the experiment, with wells H1 and H2 showing
309 reduction at lower rates. The 'low denitrification potential' well L5 showed high NO_3^-
310 reduction and this well was an intermediary between 'low denitrification potential'
311 and 'high denitrification potential' wells.

312

313 Dissolved oxygen in ‘high denitrification potential’ wells were generally below 5 mg
314 L⁻¹, with well L1 (WC) also falling within the low DO group. As a result of low DO,
315 Fe and Mn in these wells can dissolve more readily. Fe and Mn concentration was
316 highest in these wells (> 0.01 mg L⁻¹). In the ‘low denitrification potential’ wells, DO
317 ranges varied considerably, and Fe and Mn were generally at this 0.01 mg L⁻¹
318 concentration (Table 3). The pH ranges for the wells in this study ranged from 5.5 to
319 7.5. Conductivity and temperature ranged from 200 to 500 $\mu\text{S cm}^{-1}$ and from 10°C to
320 15°C, respectively. Mean concentration of DOC in all wells, except well L3 (WC),
321 was below 5 mg L⁻¹.

322

323 *3.3 Identification of covariates of importance in predictive models for NO₃-N,* 324 *Chloride, N₂/Ar and DOC.*

325 Covariates were tested and K_{sat} and water level in the well at the time of sampling
326 were found to be important and required for unbiased estimation of NO₃-N, Cl, N₂/Ar
327 and DOC. Plotting of the responses (NO₃-N, Cl, N₂/Ar and DOC) against K_{sat}
328 confirmed its importance, and the water level in the well at time of sampling proved
329 important for only some variables (i.e. NO₃-N and TOC). For a K_{sat} adjusted test of
330 the treatment effect (C enhanced wells versus all other wells), an ANOVA-type model
331 was used with those values of K_{sat} that were replicated in the observations (one for
332 each well). This gave the most general adjustment for K_{sat} fitted as a categorical
333 covariate. For NO₃-N, K_{sat} and a quadratic effect of water level were found to be
334 important ($p < 0.0001$ and $p = 0.02$ respectively), but there was no evidence for a C
335 enhancement treatment effect ($p = 0.09$). This relationship applies to all wells as
336 covariates were fitted to an analysis model containing the experiment structure
337 (treatment and day). Therefore, data from wells with and without C enhancement are

338 included. Where a covariate is significant, this adds some explanation over and above
339 such structural factors. Although the denitrification potential in the C enhanced wells
340 changed, the nitrate removal was small during the study period.

341

342 The observed means, adjusted for covariates, for NO₃-N were 4.8 mg L⁻¹ for
343 woodchip wells (L1 (WC) and L3 (WC)) and 4.0 mg L⁻¹ all other wells in the ‘low
344 denitrification potential’ category. Interpretation of the outcome for the treatment is
345 not straightforward as replication of the woodchip addition was limited to two, but the
346 outcome of the test could be regarded as marginally significant.

347 From a previous study (Fenton et al., 2009), it was clear that K_{sat} is important to
348 explain the spatial pattern of NO₃-N in glaciated till subsoils, therefore it was
349 necessary to include it in the exploratory analysis models to reduce the danger of
350 spurious relationships emerging for other factors. In order to use all the K_{sat}
351 information, a nonlinear regression was used to fit K_{sat} . Smooth curves proved
352 unsatisfactory because of the small number of K_{sat} values (this parameter does not
353 change over time in saturated conditions and is, therefore, limited to the number of
354 well screens used within an experiment i.e. eight) relative to the number of parameters
355 required, and, ultimately, a broken straight line fit was used to capture the
356 information. Having accounted for K_{sat} in this way, a significant fit for a curved
357 (quadratic) relationship between NO₃-N and water level (likelihood ratio test,
358 $p=0.007$) was found. As there is no underlying theoretical relationship in the nonlinear
359 model, its form is not expected to apply generally beyond this experiment, but the
360 model serves to highlight interesting facets of the relationships identified. Fig. 4
361 shows a plot of the predicted surface defined by K_{sat} and water level for day 54 for
362 wells L1 (WC) and L3 (WC). A plot for untreated wells or any other day would be

363 parallel to this. For CI, the ANOVA-type analysis showed no treatment effect
364 ($p=0.18$) and no effect of the covariates tested. The analysis for N_2/Ar showed no
365 effect of the treatment, but a clear quadratic relationship with K_{sat} ($p=0.44$ and
366 $p=0.007$, respectively). There was no evidence that water level played a role in
367 explaining the observations for this ratio. The decreasing magnitude of this ratio with
368 increasing K_{sat} (raw data in Fig. 5) appears to reflect the behaviour of NO_3-N . In this
369 case, a nonlinear fit does not offer any more information. For the natural logarithm of
370 DOC, there was a significant treatment effect ($p=0.04$) with both K_{sat} and water level
371 non-significant. The back-transformed, bias-corrected DOC means were 2.9 mg L^{-1}
372 for woodchip amended wells and 1.85 mg L^{-1} for other wells.

373

374 **4. Discussion**

375 *4.1. N_2/Ar ratio after carbon amendment.*

376 Agriculture, specifically intensively grazed grassland, receiving high loads of organic
377 and inorganic forms, is prone to elevated NO_3^- losses. Molecular N_2 is a benign end
378 product of denitrification, and completes the N cycle in terrestrial and aquatic
379 ecosystems. The interactive effects of C and N sources are important to reduce the
380 knowledge gap associated with denitrification potential in soils, subsoil and
381 waterbodies. The identification of how denitrification changes spatially in subsoil will
382 have consequences for groundwater and surface water protection (Hill et al., 2004).
383 Such information allows the identification of areas where natural NO_3^- reduction in
384 subsoils can actually protect a waterbody (Khalil and Richards, 2010).

385

386 Based on chemical stoichiometric relationships, denitrification of one mole of NO_3^-
387 will require 1.25 moles of C. This equates to a mass balance of 1.07 kg of available C
388 per 1 kg of NO_3^- . With approximately 50% of C availability in woodchip (based on

389 bulk density), the treatment of 1 kg of NO_3^- will be approximately 2 to 2.5 kg of
390 woodchip (Fahner, 2002). The woodchip amount used in the current study was
391 considerably less at 10 g and was still limiting, therefore, N_2/Ar ratios with higher
392 peaks could be expected in a bioreactor study. As woodchip amounts used were small,
393 denitrification potential remained similar to 'high denitrification potential' wells
394 throughout the experiment. Even at this low amendment rate, the denitrification
395 potential changes were significant. The use of natural abundance and denitrification
396 potential techniques could be used to track the NO_3^- removal efficiency of the reactive
397 media over time. A drop in denitrification potential and NO_3^- removal due to
398 denitrification could indicate a replenishment of media is needed.

399

400 Although redox conditions are favourable for denitrification processes in shallow
401 groundwater and riparian areas, there is very little denitrification potential data in the
402 literature (Ross, 1995). In particular, such data is lacking for saturated zones within
403 glaciated subsoils and C enhanced subsoils. On glaciated till subsoils (maximum
404 depth of 0.7 m), a mean N_2/Ar ratio of 40 was found during a C-enhanced incubation
405 study (Khalil and Richards, 2010). In the present study, samples were taken at an
406 average depth of 3 m and N_2/Ar ratios ranged from background (37.6) to 45. Such
407 results were comparable to a nearby shallow groundwater investigation carried out
408 within heterogeneous glacial tills (Fenton et al., 2009). In that study, N_2/Ar ratios
409 ranged from background to 44 at a mean depth of 3.6 m. Jahangir et al. (2010) found
410 N_2/Ar ranges from 40.7 to 52.0, 39.0 to 48.1, 38.5 to 41.9 and 37.8 to 40.4 in subsoils
411 of the present study site, poorly drained grassland site, sand and gravel tillage site and
412 free draining grassland site underlain by limestone, respectively. Wilson et al. (1990)
413 identified the following sequence of N_2/Ar ratios within the Lincolnshire limestone

414 aquifer: atmospheric equilibrium: $N_2/Ar = 38$, extra air entrainment: $N_2/Ar = 41$ and
415 denitrification: $N_2/Ar = 42$ to 55.

416

417 *4.2. Conditions found in aquifers for denitrification to occur.*

418 Heterotrophic denitrification is controlled by the concentrations of oxygen, NO_3^- and
419 C in shallow groundwater. Where high NO_3^- concentrations are present in such a
420 waterbody (e.g. below an agricultural landscape), the availability of degradable C
421 becomes critical for denitrification to occur. Aerobic microorganisms utilise this labile
422 C to consume oxygen. Huge spatial and temporal variation in N_2O measurements
423 from soil have been found due to changes in soil and subsoil properties such as
424 availability of mineral N (which is a substrate for nitrification and denitrification),
425 oxygen, water content, temperature, pH, redox conditions and the availability of labile
426 carbon (Chapuis-Lardy et al., 2007). In the current study, isotopes identified organic
427 fertilizer as the source of pollution on site from dairy dirty water irrigation (Table 1).
428 After removal of such a point pollution source, on site NO_3^- concentration is
429 maintained though mineralisation in the soil. While N inputs are responsible for NO_3^-
430 concentrations, the proportion of N to be denitrified is controlled by the hydrology
431 and geomorphology (Seitzinger et al., 2006). The kinetics of denitrification at N
432 concentrations $>1 \text{ mg L}^{-1}$ are independent of concentration. This implies that the rate
433 of denitrification is limited by donor availability. Carbon limitation is evident in the
434 low denitrification potentials of all wells on the site. Total organic carbon
435 concentration decreases with soil depth on site. Ryan et al. (1998) found soil and
436 subsoil denitrification rates varied spatially and temporally soil depths due to the
437 changes in soil moisture and soil NH_4^+-N . Such facts lead to differential

438 denitrification potentials with depth both spatially and temporally, and may be the
439 controlling factor for varied groundwater NO_3^- recharge concentrations.

440

441 As the oxygen levels decrease, the pH becomes more acidic, metals (micronutrients
442 Fe and Mn) dissolve, and denitrifying organisms utilise the remaining C as an electron
443 donor. Rivett et al. (2008) found denitrification occurs in aquifers when DO is 2 mg L^{-1}
444 or less, and heterotrophic denitrifying bacteria prefer a pH range from 5.5 to 8.0.
445 Significant denitrification only occurred in the current study below this DO
446 concentration, which is agreement with many studies dealing with agricultural derived
447 NO_3^- plumes (full list in Rivett et al., 2008). The DO values in all wells were much
448 lower than 2 mg L^{-1} , which shows that DO is not limiting for denitrification to occur.
449 The optimum temperature for denitrification to occur is from 25°C to 30°C , but
450 studies show a larger range from 2°C to 50°C (Rivett et al., 2008). Bailey et al.
451 (submitted) argued that low temporal variability in NO_3^- on this site was due to
452 rainfall distribution throughout the year being constant, and a temperate climate
453 allowed microbial process of nitrification and denitrification to continue all year
454 round.

455

456 The woodchip used in this study degraded rapidly increasing the DOC concentrations
457 in the C-enhanced wells. With high NO_3^- concentrations also present in these wells,
458 the denitrification potential increased turning such wells from a 'low denitrification
459 potential' signal to a 'high denitrification potential' signal. Laboratory column tests
460 have shown that DOC released from aged woodchips of two years can deplete O_2 in
461 DO saturated water in 1 hour (Robertson, 2010). In preliminary batch experiments on
462 this site, the DOC release in 24 h reached 50 mg L^{-1} in shaken tests and 40 mg L^{-1} in

463 unshaken tests. In the field, DOC levels of 15 mg L⁻¹ and 14 mg L⁻¹ were found in
464 wells L3 (WC) and L1 (WC), respectively, in the same period. Rivett et al. (2008)
465 found that DOC in most aquifers is < 5 mg L⁻¹. DOC is first oxidised by DO in the
466 system and any remaining DOC can fuel denitrification. It takes 1 mg C L⁻¹ to convert
467 2.7 mg O₂ L⁻¹. Below 1 mg O₂ L⁻¹ denitrification can occur. Dissolved organic carbon
468 requirements to fuel denitrification in each well are presented in Table 3. Dissolved
469 organic carbon deficiencies are present in ‘low denitrification potential’ wells L2 and
470 L4. Before C enhancement, well L1 (WC) and L3 (WC) had DOC concentrations of
471 1.1 and 1.4 mg L⁻¹, respectively, and had a ‘low denitrification potential’ signal. After
472 solid C enhancement, these wells had enough DOC to fuel denitrification.

473

474 4.3. K_{sat}

475 In the saturated zone, K_{sat} remains constant at a particular location but varies spatially,
476 due to the heterogeneity of the aquifer, and between different aquifers and geological
477 units. It may also vary due to anisotropies in the aquifer. The K_{sat} of glaciated tills
478 varies considerably e.g. sandy silty tills in Scandanavia range from 5 x 10⁻⁹ to 5 x 10⁻⁴
479 m s⁻¹ (Lind and Lundin, 1990). Clay tills have very low permeability of < 10⁻⁹ m s⁻¹
480 or, in some Canadian examples, vary from 10⁻¹¹ to 10⁻¹² m s⁻¹. Areas of naturally high
481 or enhanced denitrification potential, referred to as ‘denitrification hotspots’, may in
482 part be due to differential K_{sat} , mobile fractions of groundwater and slow diffusion
483 into the immobile fraction where denitrifiers are active (Schipper et al., 2005). The
484 extent to which K_{sat} controls such processes is unknown. On a mature glacial till field
485 site, Fenton et al. (2009) found that K_{sat} was positively related to mean shallow
486 groundwater (< 10 m below ground level) NO₃⁻ concentration, i.e. areas with higher
487 K_{sat} values have less time for denitrification to occur and vice versa. Gurwick et al.

488 (2008) reported that low K_{sat} areas can also be associated with buried organic matter
489 in riparian soil, which transmits water more slowly. These buried layers, in turn,
490 provide a supply of organic matter as an energy source for denitrification. In an
491 enhanced denitrification bioreactor scenario, a solid C source is mixed with soil,
492 which decreases subsurface K_{sat} in the trench. This may occur during construction
493 when compaction or insufficient mixing of soil and C material occurs. The interface
494 between the soil and the denitrification trench may also create preferential flow paths
495 into the reactive media. However, lower K_{sat} zones may establish 'denitrification
496 hotspots' (Parkin, 1987; Jacinthe et al., 1998; Schipper et al., 2005). Another
497 interesting aspect of K_{sat} related research is how it changes within a denitrification
498 wall over time, due to degradation of reactive material and/or changes in overburden
499 (Schipper et al., 2010). Interestingly, high K_{sat} may reduce denitrification due to lower
500 retention times but also it can increase recharge rate, C availability and O₂ supply.
501 Based on K_{sat} , uniform hydraulic gradient (1%) and N removal due to denitrification
502 in each well, the volume of water treated during the duration of the experiment varied
503 in each well as follows: L1 (WC) - 42 L, L2 - 21 L, L3 - (WC) 91 L, L4 - 91 L, L5 -
504 70 L and H1-H3 - 21 L. Such volumes corresponded with low N removal rates of
505 0.90, 0.01, 3.20, 0.00, 0.70, 1.08, 0.74 and 2.70 mg NO₃ m³ for the duration of the
506 experiment passing through a bioreactor 0.02 m in diameter, respectively.

507

508 Fenton et al. (2009) showed that mean groundwater NO₃⁻ was significantly ($p < 0.05$)
509 related to groundwater N₂/Ar ratio, redox potential, DO and N₂, and was close to
510 being significant with N₂O ($p = 0.08$). In this study, both spatial and temporal data was
511 utilised for NO₃⁻ prediction, K_{sat} and a quadratic effect of water level were both
512 statistically significant. When amended and no amended wells were compared, this

513 was marginally significant. Other good relationships were found between
514 denitrification potential (N_2/Ar ratio) and K_{sat} .

515

516 **5. Conclusions**

517 1. Isotopic ratios can be used to select indicative source and transformational
518 processes responsible for NO_3^- occurrence in a well, whereas N_2/Ar ratios can
519 differentiate such wells into ‘high denitrification potential’ and ‘low denitrification
520 potential’ categories. Saturated hydraulic conductivity (K_{sat}) can divide these wells
521 further into different permeability classes.

522 2. Groundwater samples retrieved using impermeable tubing and a syringe can be
523 analysed quickly, whilst avoiding de-gassing for N_2/Ar ratios using membrane inlet
524 spectroscopy (MIMS). Using this method, the denitrification potential of ‘low
525 denitrification’, ‘high denitrification’ and ‘enhanced denitrification’ wells can be
526 tracked spatially and temporally.

527 3. Data generated can then be used to develop site specific relationships and
528 predictive models for nitrate, chloride, dissolved organic carbon and N_2/Ar ratios.

529 4. Such a methodology could be applied to monitored denitrifying bioreactors to
530 identify denitrification hotspots and to examine the denitrification potential of reactive
531 media over time.

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540

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678 **Captions for Figures**

679 **Fig 1.** Schematic of Dairy farm site with location of woodchip (WC) wells, wells with
680 high (H) and low (L) denitrification potential, NO_3^- ranges and K_{sat} for each well.

681 **Fig 2.** Groundwater N_2/Ar ratios, $\text{NO}_3\text{-N}$ (mg L^{-1}) and Cl (mg L^{-1}) concentrations for
682 low (L1-5) and high (H1-H3) denitrification potential wells over time. WC here
683 signifies presence of woodchip.

684 **Fig 3.** A and B Cumulative Excess N_2 in low and high denitrification potential wells

685 **Fig 4.** Predicted $\text{NO}_3\text{-N}$ model using K_{sat} and water level.

686 **Fig 5.** Relationship between physical (K_{sat}) and denitrification (N_2/Ar ratio)
687 parameters

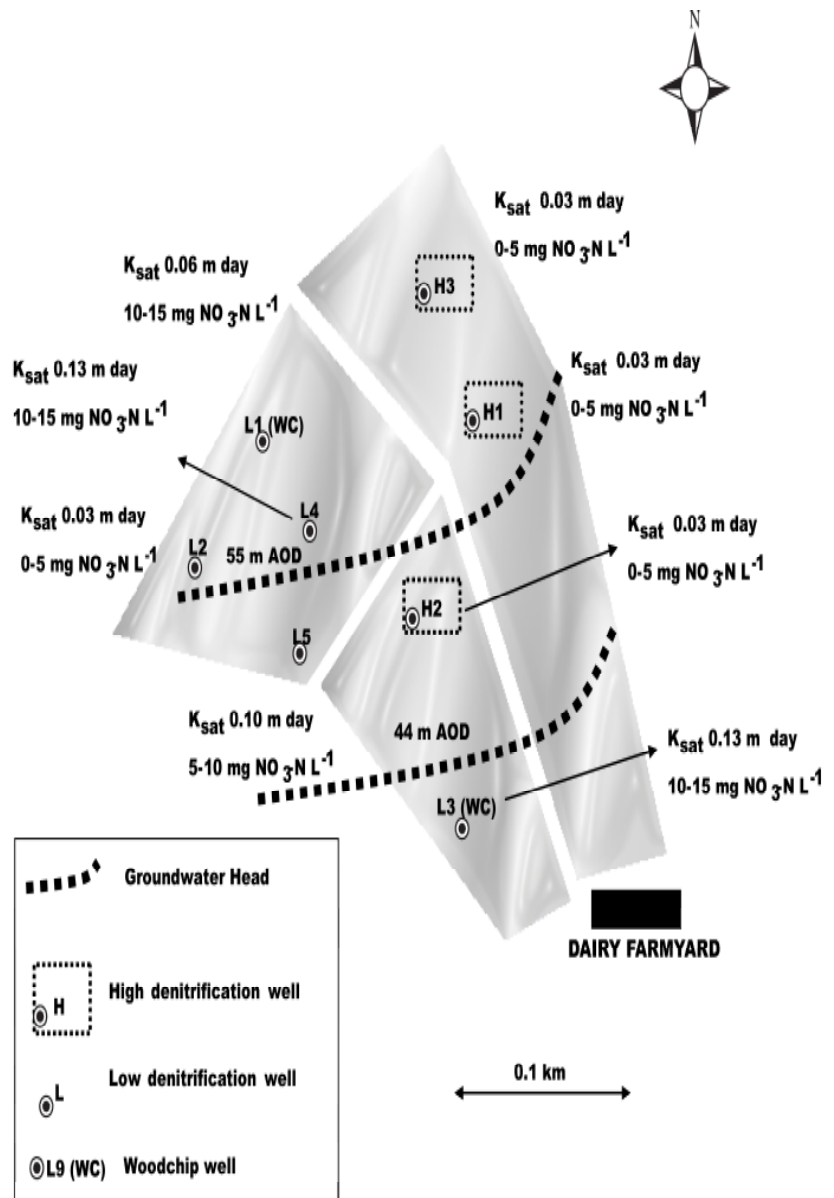
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689 **Captions for Tables**

690 **Table 1** Background isotopic data, source identification and processes in each well
691 chosen for the present study.

692 **Table 2** Nitrate, chloride and N_2/Ar min, max and mean for all wells during the study
693 period.

694 **Table 3.** DO, Fe, Mn, DOC and mean denitrification data for all wells during the
695 study.



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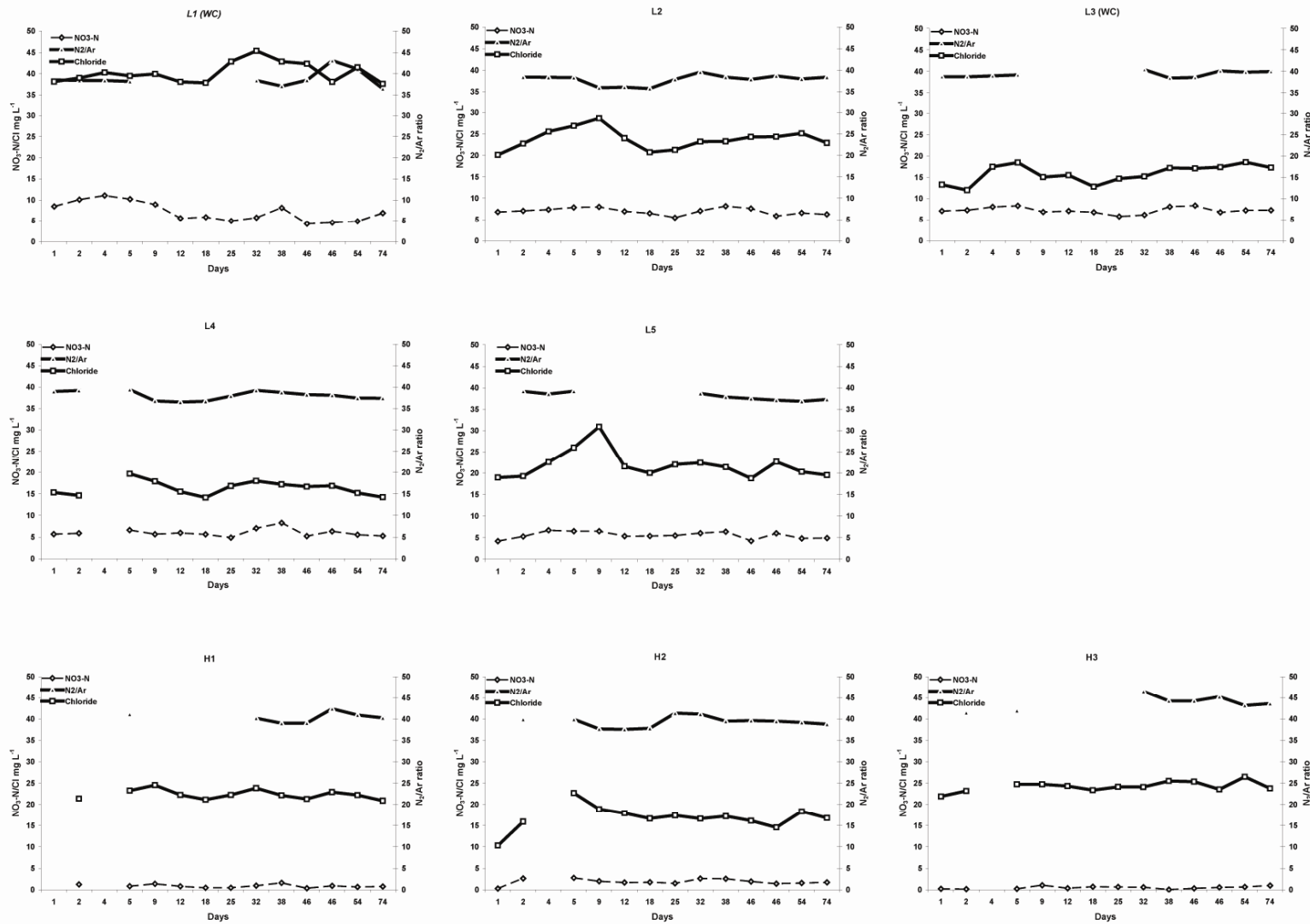
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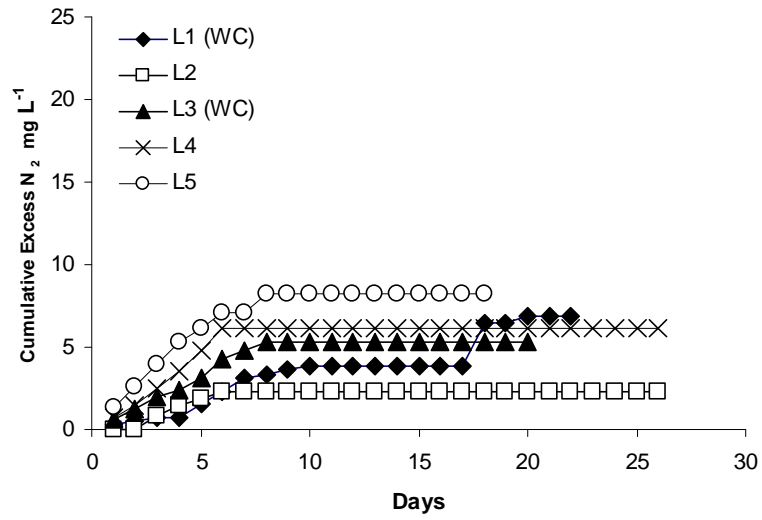
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720 **Fig 2.** Groundwater N_2/Ar ratios, NO_3-N ($mg L^{-1}$) and Cl ($mg L^{-1}$) concentrations for low (L1-5) and high (H1-H3) denitrification potential wells
721 over time. WC here signifies presence of woodchip.

A) 'Low denitrification potential' wells



B) 'High denitrification potential' wells

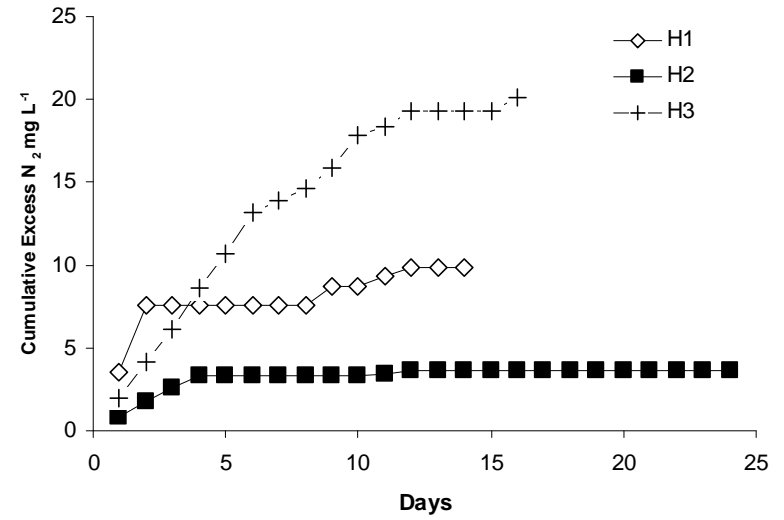


Fig 3. A and B Cumulative Excess N_2 in low and high denitrification potential wells

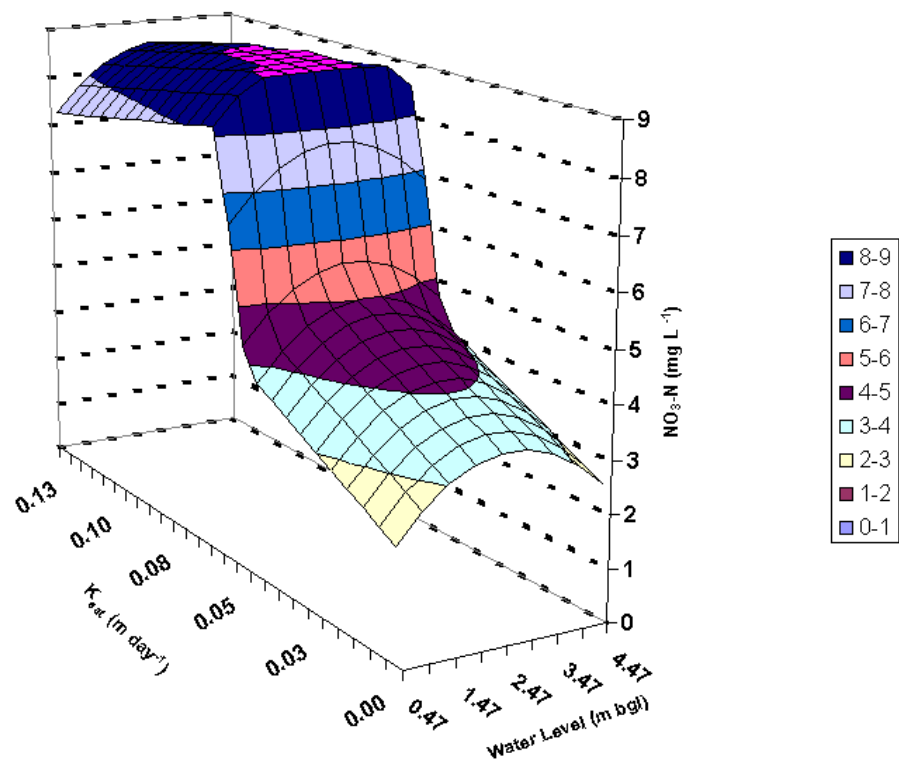


Fig 4. Predicted $\text{NO}_3\text{-N}$ model using K_{sat} and water level.

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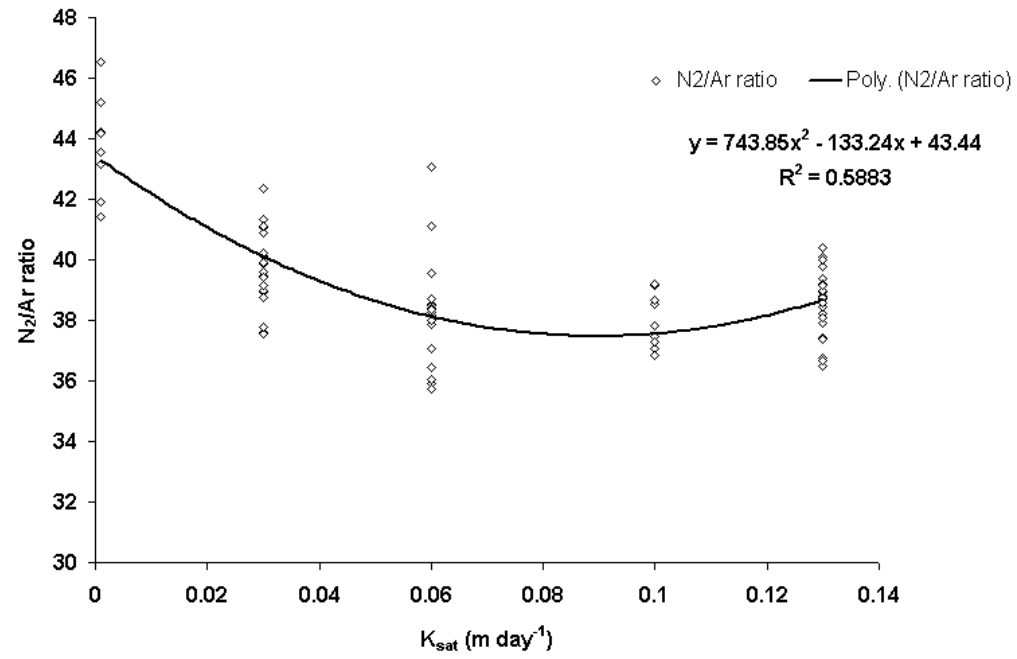


Fig 5. Relationship between physical (K_{sat}) and denitrification (N_2/Ar ratio) parameters.

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Table 1 Background isotopic data, source identification and processes in each well chosen for the present study.

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Well	NO ₃ -N	δ ¹⁵ N	δ ¹⁸ O	NO ₃ -N	δ ¹⁵ N	δ ¹⁸ O	NO ₃ -N	δ ¹⁵ N	δ ¹⁸ O	Source of Nitrate	Transformational Process
	mg L ⁻¹	‰	‰	mg L ⁻¹	‰	‰	mg L ⁻¹	‰	‰		
	April 2008			August 2008			December 2008				
<i>L1 WC</i>	9.8	6.8	5.3	12.9	7.1	5.7	12.7	8.9	8.7	Manure	Nitrification but no denitrification
L2	8.4	7.3	4.6	8.5	6.7	4.1	8.6	7.7	5.6	Manure	Nitrification but no denitrification
<i>L3 WC</i>	12.9	6.8	1.4	13.0	6.6	1.4	10.7	7.3	3.7	Low manure signal	Nitrification but no denitrification
L4	13.5	7.6	5.4	11.4	7.7	4.8	11.1	8.2	9.6	Manure	Nitrification but no denitrification
L5	7.3	9.1	8.1	7.6	7.8	4.4	7.3	8.4	5.7	Manure/sewage	Soil nitrification
H1	3.7	9.2	6.1	1.8	11.4	8.1	0.8	11.7	7.9	Manure	Denitrification
H2	4.2	11.7	9.6	3.3	13.1	11.6	2.6	16.2	14.7	Manure	Lots of denitrification
H3	0.3	7.6	4.6	0.3	16.2	14.2	0.1	16.7	16.1	Manure	High nitrification, volatilisation and denitrification

Table based on Baily et al. (submitted)

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Table 2 Nitrate, chloride and N₂/Ar min, max and mean for all wells during the study period.

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Well	WT ^b	Screen ^c	Nitrate	Nitrate	Nitrate	Chloride ^a	Chloride	Chloride	N ₂ /Ar	N ₂ /Ar	N ₂ /Ar	DOC	TOC
	Mean		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Mean	Mean
	m bgl	m bgl	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	ratio	ratio	ratio	mg L ⁻¹	mg L ⁻¹
‘low denitrification potential’ wells													
L1 (WC)	2.6	3.4	4.2	11.0	7.1	37.6	45.3	40.2	36.4	43.0	38.8	2.9	30.9
L2	2.9	5.9	5.4	8.1	6.9	20.0	28.6	23.8	35.7	39.5	37.8	1.2	7.1
L3 (WC)	2.5	4.9	5.7	8.3	7.1	11.8	18.5	15.8	38.5	40.4	39.3	7.0	57.1
L4	1.3	3.0	4.8	8.2	5.9	14.1	19.6	16.3	36.5	39.4	38.0	1.4	6.2
L5	4.6	7.8	4.1	6.7	5.5	18.7	30.8	21.6	36.8	39.2	38.0	3.2	12.8
‘high denitrification potential’ wells													
H1	7.4	8.1	0.3	1.5	0.8	20.8	24.5	22.3	39.0	42.4	40.4	1.7	5.8
H2	0.9	2.5	0.2	2.7	1.8	10.3	22.6	16.8	37.5	41.3	39.3	1.4	6.6
H3	4.0	4.5	0.003	1.0	0.4	21.9	26.5	24.2	41.4	46.5	43.8	1.9	4.7

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^aIn Ireland natural background levels of Chloride in groundwater are 18 mg L⁻¹. Concentrations above this are due to influences from the pollution sources on the far^bMean Watertable Height^cTop of screen, total Well depth + 1m

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Table 3. DO, Fe, Mn, DOC and mean denitrification data for all wells during the study.

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Well	DO ^a mg L ⁻¹	Fe ^b mg L ⁻¹	Mn ^c mg L ⁻¹	DOC ^d mg L ⁻¹	DOC ^e mg L ⁻¹	Mean Excess N ₂ mg L ⁻¹	Reaction Progress Ratio
'low denitrification potential' wells							
L1 WC	0.6	3.4	0.7	1.2	2.9	0.55±0.30	0.05±0.06
L2	1.7	10.5	0.0	3.8	1.2	0.02±0.13	0.01±0.03
L3 WC	0.3	5.2	0.0	1.9	7.3	0.88±0.12	0.06±0.06
L4	0.7	8.8	0.0	3.2	1.4	0.00±0.12	0.00±0.02
L5	0.5	6.8	0.0	2.5	3.2	0.25±0.18	0.03±0.05
'high denitrification potential' wells							
H1	0.4	0.9	0.0	0.3	1.7	1.29±0.20	0.26±0.26
H2	0.3	4.0	0.0	1.4	1.4	0.89±0.13	0.18±0.18
H3	0.3	4.8	0.2	1.7	1.9	3.22±0.28	0.90±0.91

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^amin, ^bmax, ^cmean, ^drequired for denitrification, ^eavailable for denitrification.