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23 **Evaluation of headspace equilibration methods for quantifying**
24 **greenhouse gases in groundwater**

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41 **Abstract**

42 The objective of the study was to evaluate the different headspace equilibration methods for
43 the quantification of dissolved greenhouse gases in groundwater. Groundwater samples were
44 collected from wells with contrasting hydrogeochemical properties and degassed using the
45 headspace equilibration method. One hundred samples from each well were randomly selected,
46 treatments were applied and headspace gases analysed by gas chromatography. Headspace
47 equilibration treatments varied helium (*He*): water ratio, shaking time and standing time. Mean
48 groundwater N₂O, CO₂ and CH₄ concentrations were 0.024 mg N L⁻¹, 13.71 mg C L⁻¹ and 1.63
49 µg C L⁻¹, respectively. All treatments were found to significantly influence dissolved gas
50 concentrations. Considerable differences in the optimal *He*: water ratio and standing time were
51 observed between the three gases. For N₂O, CO₂ and CH₄ the optimum operating points for
52 *He*: water ratio was 4.4:1, 3:1 and 3.4:1; shaking time was 13, 12 and 13 min; and standing
53 time was 63, 17 and 108 min, respectively. The headspace equilibration method needs to be
54 harmonised to ensure comparability between studies. The experiment reveals that *He*: water
55 ratio 3:1 and shaking time 13 min give better estimation of dissolved gases than any lower or
56 higher ratios and shaking times. The standing time 63, 17 and 108 min should be applied for
57 N₂O, CO₂ and CH₄, respectively.

58 Key words: greenhouse gas, headspace extraction, dissolved gases, shaking time, standing time

59

60 **Introduction**

61 Measurement of dissolved gases in groundwater is becoming increasingly more common for
62 the estimation of greenhouse gas emissions and understanding biogeochemical cycles. Methane
63 (CH₄), nitrous oxide (N₂O) and carbon dioxide (CO₂) are important greenhouse gases (GHGs)
64 that contribute to global warming (Ferron et al., 2007). Indirect N₂O emissions are now

65 recognized as a quantitatively significant component of the total N₂O emission budget from
66 agricultural activities (Reay et al., 2009). The IPCC (2006) uses the default emission factor for
67 the indirect N₂O emission associated with groundwater (EF_{5g}) of 0.0025. Indirect N₂O
68 emissions from drainage water can account for 50 to 67% of the above ground direct emissions
69 (Minamikawa et al., 2010).

70 Current studies have shown that CO₂ and CH₄ are also emitted via indirect pathways.
71 Dissolved CO₂ in river water is an important component of the terrestrial carbon cycle and an
72 important pathway for CO₂ emission to the atmosphere (Minamikawa et al., 2010).
73 Groundwater in the UK was an important source of dissolved CO₂ in the rivers (Worrall and
74 Lancaster, 2005). Stream flow in the Amazonian headwater catchment was predominantly
75 derived from deeper flow paths containing water with high dissolved CO₂ concentrations
76 (Johnson et al., 2006). High CH₄ concentrations in groundwater have been observed in the four
77 major UK aquifers where groundwater had the highest reductive potential (Goody and Darling,
78 2005). The concentration gradient of dissolved CH₄ between ambient air and water bodies
79 contributes to substantial CH₄ emission from groundwater to the atmosphere (Sawamoto et al.,
80 2002). Large quantities of predominantly biogenic CH₄ δ¹³C (-72.1±6.8‰) and δ²H (-
81 297±17‰) have been reported to occur from CO₂ reduction in shallow groundwater in Canada
82 (Cheung et al., 2010). Therefore, quantification of dissolved GHGs in surface water,
83 groundwater, drainage water, pore water etc. are important in evaluating biogeochemical
84 transformations in subsurface soils and sediments contaminated with nitrate and petroleum
85 fuels, as well as evaluating the indirect sources and concentrations of major GHGs.

86 The headspace equilibration technique is a widely used method for extraction of dissolved
87 gases in water due to its simplicity, reliability and adaptability to routine analysis of samples
88 (Kampbell et al., 1989). The technique, although widely adopted, has been applied with

89 diverging ratios of *He*: water (v/v) and shaking times for the equilibration of dissolved gases
90 between the liquid and headspace phases. For example, Geistlinger et al. (2010) measured
91 dissolved N₂O, CO₂ and CH₄ in groundwater with 1:1 headspace to water ratio and shaken for
92 2 h. Hamilton and Ostrom (2007) extracted groundwater dissolved gases at a ratio (*He*: water)
93 of 2:1 (v/v) and vigorously agitated for 5 min. Conversely, Clough et al. (2007) degassed water
94 with headspace: water = 7.:1 (v/v) and shaken for 1 min. Reay et al. (2003) analysed dissolved
95 gases in drainage water using an empty space: water ratio of 3.4:1 and vigorously shaken for 2
96 min followed by a 30 min standing period. However, Kellogg (2005) analysed groundwater for
97 dissolved gases (N₂, N₂O, ¹⁵N, ¹⁵N₂O, SF₆) using a headspace: water ratio of 6.5:1. In drainage
98 water from lysimeters, Minamikawa et al. (2010) measured annual emissions of GHGs (N₂O,
99 CO₂ and CH₄) using *He*: water ratio of 1:1 (v/v) and vigorously shaken for 1 min. The
100 variation in the application of the headspace equilibration technique, with numerous ratios and
101 shaking times, raises the issue of the comparability of results between studies.

102 We hypothesise that *He*: water ratio, shaking time and standing time influence the equilibration
103 of gases dissolved in ground/surface water effecting the absolute concentrations observed. The
104 objectives of the study were to (i) evaluate the effects of *He*: water ratio, shaking times and
105 standing period on GHG extraction and (ii) examine if the treatment effects vary with the
106 background dissolved gas concentration.

107

108 **Materials and methods**

109 2.1 Groundwater sampling and analysis

110 Groundwater sampling was carried in 3 monitoring wells (0.05 m ID; 2.0 m screen section)
111 installed at 5 m below ground level (BGL) in a transect along groundwater flow path under
112 intensively managed grazed grassland in Southeast Ireland (52°20'3"N, 6°27'27"W). Each well

113 was approximately 300 m apart with contrasting hydrogeochemical properties and dissolved
114 gas concentrations of GHGs which are summarized in Table 1. A total of 300 samples were
115 collected using a bladder pump (Geotech Environmental Equipment, Inc., USA) following
116 USEPA Region I Low Stress Purging and Sampling Procedures (USEPA, 1996). Samples were
117 collected from the teflon pump outlet tube (ID 0.006 m) at a rate of 100 ml min⁻¹ to prevent
118 ebullition of dissolved gases. Water samples were collected in 160 ml glass serum and bottles
119 sealed immediately with butyl rubber septa and aluminium crimp caps (WHEATON, USA).
120 No visible air bubbles were observed inside the bottle. All samples were submerged below
121 water in a cool box, stored at 4 °C and analysed within one week. Treatments were randomly
122 assigned to samples from each well to examine the effects of headspace: water ratio, shaking
123 time and standing time on dissolved GHGs. The experimental treatments are listed below:

124 a. Five *He*: water ratios (v/v): R1 (3:1), R2 (1.5:1), R3 (1:1), R4 (1:1.5) and R5 (1:3);

125 b. Five shaking times: S1 (0 min), S2 (1 min), S3 (5 min), S4 (10 min) and S5 (20 min);

126 and

127 c. Four standing times: T1 (0 min), T2 (15 min), T3 (30 min) and T4 (60 min).

128 Samples were degassed using high purity *He* (BOC, Linde Group, Germany). The required
129 headspace volume was augmented to 120, 96, 80, 64 and 40 ml by injecting *He* and replacing
130 water simultaneously through the rubber septum of sealed serum bottle using hypodermic
131 needle and polyvinyl syringe resulting in the *He*: water ratios of R1, R2, R3, R4 and R5. The
132 samples were shaken on a gyrotory shaker (Model G-10, New Brunswick Scientific Co.,
133 USA) at 400 rpm for the required time as per the treatments levels S1-S5. Samples were then
134 left undisturbed at 21° C for the required times T1-T4. After equilibration, a headspace gas
135 sample was collected in a 12 ml exetainer (Labco, Wycombe, UK) with an additional injection
136 of 15 ml *He* using a PVC syringe and the dilution factor was taken into consideration during

137 the calculation of dissolved gases. Sample degassing, shaking and headspace gas extraction
138 were done at 21° C. N₂O, CO₂ and CH₄ were quantified by gas chromatography (CP-3800 GC,
139 Varian, Inc. USA/CTC Analytics combi PAL Auto Sampler, Switzerland) equipped with an
140 electron capture detector (ECD), a thermal conductivity detector (TCD), and a flame ionization
141 detector (FID) using Ar as a carrier gas. The GC has a Porapak-Q column (80-100 MESH),
142 3.7m x 1/8" x 2.0 mm. Calibration of the GC system was conducted using a seven
143 concentrations of each standard gas. The precision of analysis was satisfactory with
144 coefficients of variation between analyses of 0.3%.

145

146 2.2 Estimation of dissolved GHGs and hydrochemical analysis

147 The N₂O, CO₂ and CH₄ concentrations in water samples were estimated using Henry's law
148 constant, headspace gas concentrations, bottle volume and groundwater recharge temperature
149 at sample collection. The partial pressures of N₂O, CO₂ and CH₄ in the equilibrated headspace
150 and water were calculated using solubility of gases from Weiss and Price (1984) for N₂O,
151 Weiss (1974) for CO₂ and Wilhelm et al. (1977) for CH₄ at the recharge temperature as
152 measured at the interface between the unsaturated zone and groundwater surface. Groundwater
153 non-metallic ions e. g. total oxidised N and NO₂⁻ were analyzed by Aquakem 600 Discrete
154 Analyser (Aquakem 600A, 01621 Vantaa, Finland).

155

156 2.3 Statistical analysis

157 Regression analysis was used to identify the optimum equilibration variables. For regression,
158 the experiment factors were treated as continuous variables and the MIXED procedure (SAS,
159 2009) was used for the formal analysis of significant effects. Response-surface analysis was
160 used to examine the general effects of the treatment variables and to establish optimal

161 operating points for them. The RSREG procedure (SAS, 2009) was used to fit a quadratic
162 response model and to plot the response surfaces with estimates of optimal treatment points.
163 Residual checks were made and responses were transformed as required to ensure that the
164 assumptions of the analysis (Normality and constant variance) were met. As the focus in the
165 analysis was on selection, if possible, of an optimum operating point, graphical methods were
166 used instead of examining the regression coefficients in detail. Plots were made of predicted
167 values of the measurement variable, showing how it varied with the experimental factors. One
168 variable was held fixed, at a range of values, while the others varied continuously, producing a
169 set of 3-dimensional plots.

170

171 **Results**

172 The response surfaces fitted for Ratio of *He*: water, shaking time and standing time indicated
173 curvature in the relationships with dissolved gases. For the N₂O results, one of the wells had
174 low concentrations, producing very low response values. [These responses were removed from](#)
175 [the analysis as it appeared to be spurious and quite different to the general behaviour across the](#)
176 [rest of the data set.](#) The range of the responses in the analysis was from 0.004 to 0.86 mg N₂O-
177 N L⁻¹. Residual checks for the N₂O results showed evidence of non-constant variance and a log
178 transformation was used to compensate for this. The formal analysis of the regression
179 relationships (Table 2) showed that there was curvature in the relationships with all the
180 experimental factors. There was a significant quadratic term for each as well as crossed terms
181 which allowed the slope of a linear relation with one variable to change smoothly as another
182 variable changed. The terms showing interactions with wells provided evidence that the
183 quadratic coefficients for standing time and shaking time varied from well to well.

184 Examination of plots of the type illustrated in Figure 1 was used to interpret the results shown
185 in Table 2.

186 The graphics indicated that there was a consistent optimum operating point for shaking time,
187 approximately in the centre of the range examined. There was a good indication that the
188 standing time was optimum or near-optimum at the high end of the range but an optimum
189 value for ratio was not covered by this data set. While there was significant curvature detected
190 in this latter functional relationship, the response continued to increase as the ratio approached
191 the maximum value of 3. Determination of the optimum, based on this data set required
192 extrapolation. The RSREG procedure identified a maximum in the fitted surface for dissolved
193 N₂O and estimated it at a *He*: water ratio = 4.4, shaking time = 13 min and standing time = 63
194 min. Plots were prepared for each well separately to check for effects of the interactions of the
195 terms with wells. The relationship with shaking time was stable across wells while well 1
196 indicated a standing time optimum within the range in the data set.

197 Dissolved groundwater CH₄ (ranging from 0.003 to 9.097 mg C L⁻¹) was not normally
198 distributed and required square root transformation. The formal analysis (Table 2) shows
199 quadratic terms for *He*: water ratio and shaking time but not for standing time. Nor does
200 standing time interact with either *He*: water ratio or shaking time. There was evidence that the
201 slope varied between wells.

202 Graphics confirmed this increasing trend across the range of standing times examined. An
203 optimum value for standing time was not covered by this data set nor was there statistical
204 evidence that an optimum existed. However, plots for standing time with curvature terms
205 included did indicate that the trend in curvature was such that there would be an optimum at
206 some greater value of standing time. Furthermore, while the data suggested that there was an
207 optimum value of ratio near the high end of the range, it was not well defined and there was a

208 linear trend that varied from well to well. An optimum for shaking time was better defined in
209 the middle of the range. A maximum in the regression equations was determined at *He*: water
210 ratio = 3.4, shaking time = 13 min and standing time = 108 min. The standing time estimate
211 was determined by the fitting non-significant curvature terms to underline that an optimum
212 point was likely to be considerably outside the range of values in this study. It was noted that
213 the estimated optimum ratio was close to the range of ratios examined.

214 Groundwater CO₂ varied from 0.19 to 54.39 mg C L⁻¹. For log CO₂, examination of the
215 reduced model in Table 2 showed that there were significant quadratic terms for ratio and
216 shaking time while standing time showed only a linear trend that varied from well to well,
217 indicating possible optima for ratio and shaking time but no evidence of an optimum for
218 standing time. Graphical representations indicated that, as for CH₄ and N₂O, there was a clear
219 optimum shaking time in the middle of the range examined with an indication that there was
220 little impact of standing time on the results (the magnitude of the slope in the linear effect was
221 small). The ratio was found to be near an optimum at the top of its range but this was not well-
222 defined. No maximum was found in the predicted response surface. When quadratic terms
223 were fitted for standing time, a saddle point, where there is a maximum for one or more
224 variables with a minimum for others, was estimated at ratio = 3, shaking time = 12 min and
225 standing time = 17 min. The standing time produced the minimum. Individual data for all
226 GHGs has been included in Figure 2, 3 and 4, as electronic supplementary material

227

228 **Discussion**

229 Initially we hypothesised that *He*: water ratio, shaking times and standing time influence the
230 equilibration of gases dissolved in ground/surface water effecting the absolute concentrations
231 observed. [All the published papers to date simultaneously vary these three treatment factors](#)

232 making it impossible to separate their individual effects. Dissolved N₂O extraction in
233 groundwater with *He*: water ratio of 1:1 by Geistlinger et al. (2010) and 1.36:1 by Von der
234 Heide et al. (2008) measured respectively 0.010 and 0.013 mg N L⁻¹ with 120 min shaking in
235 similar geochemical environments to our study in Germany. Interestingly, their results were
236 close to our results with *He*: water ratio of 1:1 (0.012 mg N L⁻¹) which increased to 0.032 mg N
237 L⁻¹ at a ratio of 3:1. Similarly, Lemon (1981), Ferron et al. (2007) and Reay et al. (2003)
238 measured dissolved N₂O concentrations in surface water with *He*: water ratios of respectively
239 1:1, 1.5:1 and 3.4:1 giving 0.0003, 0.0002 and 0.0020 mg N L⁻¹ with several days, 1 min
240 vigorous and 2 min shaking, respectively. These results indicate that headspace volume should
241 be 3 times higher than water volume to completely equilibrate dissolved N₂O; otherwise the
242 extracted concentration maybe underestimated. This is in broad agreement with our results
243 where a minimum *He*: water ratio of 3:1 was required. Similarly, the lower *He*: water ratios in
244 the current work confirmed that *He*: water ratio less than 1.5:1 underestimates the dissolved
245 groundwater N₂O concentration. Our study also highlights that the optimum shaking time for
246 degassing dissolved N₂O was 13 minutes which is longer than most of the afore mentioned
247 studies.

248 The CO₂ concentration (14.4 mg C L⁻¹) measured by von der Heide et al. (2008) with 1.36:1
249 ratio (shaking time 120 min and no standing time) was similar to our result (12.3 mg C L⁻¹) at a
250 similar ratio. Our results indicate that at the higher ratio of 3:1 the CO₂ concentration doubled
251 to 26.8 mg C L⁻¹, indicating an underestimate by Heide et al. (2008). This highlights that
252 similar to N₂O, dissolved CO₂ concentrations are underestimated at low *He*: water ratios.

253 To obtain complete equilibration in CH₄ concentrations it is clear that a *He*: water ratio of at
254 least 3:1, at least 10 minutes of shaking. Minamikawa et al. (2010) measured CH₄
255 concentration (0.36 ug C L⁻¹) with a 1:1 ratio (shaking time 1 min and no standing time) in

256 drainage water from a similar soil type and their CH₄ concentration was similar to our result
257 (0.40 ug C L⁻¹) at the same ratio. However, we found that increasing shaking time to 10
258 minutes and standing time to 1 hour resulted in CH₄ concentration more than doubled to 98 ug
259 C L⁻¹.

260 Our study showed that *He*: water ratio, shaking time and standing time are critical factors in the
261 estimation of dissolved greenhouse gases in groundwater under a range of existing
262 geochemical environments. Shaking time was found to have a consistent optimum operating
263 point in this study. The study also provided indications that the optima for the other factors are
264 greater than the highest values covered. The standing time had the most variable impact on the
265 results and it appears to vary substantially between gases and between wells. Further research
266 is recommended to test the effects of the degassing method across a wider range of
267 hydrogeological settings to standardise the headspace equilibration method.

268

269 **Conclusions**

270 The present study indicates that the *He*: water ratio, shaking time and standing time all
271 significantly effect the concentrations of N₂O, CO₂ and CH₄ in groundwater. Estimation of
272 dissolved greenhouse gases in groundwater is likely to be underestimated due to incomplete
273 gas equilibration. All treatments were found to significantly influence dissolved gas
274 concentrations. Considerable differences in the optimal *He*: water ratio and standing time were
275 observed between the three gases but shaking time was same for all (ca.13 min). The
276 experiment reveals that *He*: water ratio 3:1 and shaking time 13 min give better estimation of
277 dissolved gases than any lower or higher ratios and shaking times. The standing time 63, 17
278 and 108 min should be applied for N₂O, CO₂ and CH₄, respectively. The headspace

279 equilibration technique should be harmonised internationally to improve global predictions of
280 indirect greenhouse gas emissions and improve inter-comparability of results.

281

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286

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350 *Table 1* Hydrogeochemical properties of the wells sampled for the analyses of dissolved gases

351

Groundwater Parameter	Well 1	Well 2	Well 3
Chemical parameter (mg L ⁻¹)			
Nitrous oxide (N ₂ O-N)	0.048	0.019	0.005
Carbon dioxide (CO ₂ -C)	12.61	21.26	26.53
Methane (CH ₄ -C)	3.06	0.77	1.07
Dissolved organic carbon (DOC-C)	0.9	0.77	13.47
Nitrate-N	2.55	4.62	2.73
Physico-chemical parameter			
Dissolved oxygen (mg L ⁻¹)	2.67	3.03	4.38
pH	6.84	6.78	6.45
Redox potential (mV)	118	131	140
Hydrogeological parameter			
Groundwater table depth (m)	2.27	2.29	1.97
Saturated hydraulic conductivity (m d ⁻¹)	0.012	0.011	0.013

352

353

354 *Table 2* Tests of effects from regression analysis of N₂O, CO₂ and CH₄ results (p < 0.5 for

355 inclusion in the final model)

	N ₂ O‡	CH ₄ #	CO ₂ ‡
Effect	Pr > F	Pr > F	Pr > F
Well	<.0001	0.0079	<.0001
Ratio	0.0019	<.0001	<.0001
Shaking Time	0.0404	0.0007	<.0001
Shaking Time*Well	<.0001	<.0001	ns
Ratio*Well	<.0001	0.0176	<.0001
Standing Time	0.0049	<.0001	ns
Standing Time*Well	<.0001	<.0001	0.0053
Ratio*Standing Time	0.0104	ns	ns
Ratio*Ratio	0.0234	0.0087	0.0003
Shaking Time*Shaking Time	0.0247	0.0238	<.0001
Standing Time*Standing Time	ns	ns	ns
Standing Time*Standing Time*Well	0.0041	ns	ns
Shaking Time*Shaking Time*Well	<.0001	ns	ns

356 ‡data log-transformed;

357 #data square root-transformed;

358 ns nonsignificant

359 *Figure 1* Graphical representation of the fitted response surface for shaking time and *He*:
360 water ratio (ratio) at a fixed standing time of 60 minutes. The predicted value refers to the log
361 concentration of N₂O
362