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Title: Combining stable isotopes with contamination indicators: a method for improved investigation of nitrate sources and dynamics in aquifers with mixed nitrogen inputs

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Abstract: Excessive nitrate (NO3-) concentration in groundwater raises health and environmental issues that must be addressed by all European Union (EU) member states under the Nitrates Directive and the Water Framework Directive. The identification of NO3- sources is critical to efficiently control or reverse NO3- contamination that affects many aquifers. In that respect, the use of stable isotope ratios 15N/14N and 18O/16O in NO3- (expressed as δ15N-NO3- and δ18O-NO3-, respectively) has long shown its value. However, limitations exist in complex environments where multiple nitrogen (N) sources coexist. This two-year study explores a method for improved NO3- source investigation in a shallow unconfined aquifer with mixed N inputs and a long established NO3- problem. In this tillage-dominated area of free-draining soil and subsoil, suspected NO3- sources were diffuse applications of artificial fertiliser and organic point sources (septic tanks and farmyards). Bearing in mind that artificial diffuse sources were ubiquitous, groundwater samples were first classified according to a combination of two indicators relevant of point source contamination: presence/absence of organic point sources (i.e. septic tank and/or farmyard) near sampling wells and exceedance/non-exceedance of a contamination threshold value for sodium (Na+) in groundwater. This classification identified three contamination groups: agricultural diffuse source but no point source (D+P-), agricultural diffuse and point source (D+P+) and agricultural diffuse but point source occurrence ambiguous (D+P±). Thereafter δ15N-NO3- and δ18O-NO3- data were superimposed on the classification. As δ15N-NO3- was plotted against δ18O-NO3-, comparisons were made between the different contamination groups. Overall, both δ variables were significantly and positively correlated ($p < 0.0001$, rs = 0.599, slope of 0.5), which was indicative of denitrification. An inspection of the contamination groups revealed that denitrification did not occur in the absence of point source contamination (group D+P-). In fact, strong significant denitrification lines occurred only in the D+P+ and D+P± groups ($p < 0.0001$, rs > 0.6, $0.53 \le$ slope ≤ 0.76), i.e. where point source

contamination was characterised or suspected. These lines originated from the 2-6‰ range for δ15N-NO3-, which suggests that i) NO3- contamination was dominated by an agricultural diffuse N source (most likely the large organic matter pool that has incorporated 15N-depleted nitrogen from artificial fertiliser in agricultural soils and whose nitrification is stimulated by ploughing and fertilisation) rather than point sources and ii) denitrification was possibly favoured by high dissolved organic content (DOC) from point sources. Combining contamination indicators and a large stable isotope dataset collected over a large study area could therefore improve our understanding of the NO3- contamination processes in groundwater for better land use management. We hypothesise that in future research, additional contamination indicators (e.g. pharmaceutical molecules) could also be combined to disentangle NO3- contamination from animal and human wastes.

***Highlights (for review)**

HIGHLIGHTS

- Groundwater samples were collected from an aquifer with mixed N inputs
- N source apportionment was first based on indicators of point source contamination
- $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- were then superimposed to N source apportionment
- The dominant source of groundwater NO_3^- was characterised (agricultural diffuse)
- Conditions for denitrification were identified (point source contamination)

Combining stable isotopes with contamination indicators: a method for improved investigation of nitrate sources and dynamics in aquifers with mixed nitrogen inputs

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ABSTRACT

Excessive nitrate (NO_3^-) concentration in groundwater raises health and environmental issues that must be addressed by all European Union (EU) member states under the Nitrates Directive and the Water Framework Directive. The identification of NO_3^- sources is critical to efficiently control or reverse NO_3^- contamination that affects many aquifers. In that respect, the use of stable isotope ratios $^{15}N/^{14}N$ and $^{18}O/^{16}O$ in NO_3^- (expressed as $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-, respectively) has long shown its value. However, limitations exist in complex environments where multiple nitrogen (N) sources coexist. This two-year study explores a method for improved NO_3^- source investigation in a shallow unconfined aquifer with mixed N inputs and a long established NO_3^- problem. In this tillage-dominated area of free-draining soil and subsoil, suspected NO_3^- sources were diffuse applications of artificial fertiliser and organic point sources (septic tanks and farmyards). Bearing in mind that artificial diffuse sources were ubiquitous, groundwater samples were first classified according to a combination of two indicators relevant of point source contamination: presence/absence of organic point sources (i.e. septic tank and/or farmyard) near sampling wells and exceedance/non-exceedance of a contamination threshold value for sodium (Na^+) in groundwater. This classification identified three contamination groups: agricultural diffuse source but no point source (D+P-), agricultural diffuse and point source (D+P+) and agricultural diffuse but point source occurrence ambiguous (D+P±). Thereafter $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- data were superimposed on the classification. As $\delta^{15}N$-NO_3^- was plotted against $\delta^{18}O$-NO_3^-, comparisons were made between the different contamination groups. Overall, both δ variables were significantly and positively correlated ($p < 0.0001$, $r_s = 0.599$, slope of 0.5), which was indicative of denitrification. An inspection of the contamination groups revealed that denitrification did not occur in the absence of point source contamination (group D+P-). In fact, strong significant denitrification lines occurred only in the D+P+ and D+P± groups ($p < 0.0001$, $r_s > 0.6$, $0.53 \leq \text{slope} \leq 0.76$), i.e. where point source contamination was characterised or suspected. These lines originated from the 2-6‰ range for $\delta^{15}N$-NO_3^-, which suggests that i) NO_3^- contamination was dominated by an agricultural diffuse N source (most likely the large organic matter pool that has incorporated ^{15}N-depleted nitrogen from artificial fertiliser in agricultural soils and whose nitrification is stimulated by ploughing and fertilisation) rather than point sources and ii) denitrification was possibly favoured by high dissolved organic content (DOC) from point sources. Combining contamination indicators and a large stable isotope dataset collected over a large study area could therefore improve our understanding of the NO_3^- contamination processes in groundwater for better land use management. We hypothesise that in future research, additional contamination indicators (e.g. pharmaceutical molecules) could also be combined to disentangle NO_3^- contamination from animal and human wastes.

KEY WORDS

Nitrate; Groundwater; Stable isotope; Contamination indicator; Diffuse source pollution; Point source pollution

1 INTRODUCTION

Elevated levels of nitrate (NO_3^-) in aquifers have long been a cause of concern (Stark and Richards, 2008). If ingested, contaminated groundwater is potentially harmful to human and animal health. From an environmental perspective, discharge into surface-water bodies can contribute to eutrophic conditions in lakes, streams, estuaries and the coastal zone. Groundwater NO_3^- also represents a source of indirect nitrogen (N) losses which occur as a result of partial denitrification of NO_3^- into nitrous oxide (N_2O), a powerful greenhouse gas. Eventually, such contamination issues may have detrimental knock-on effects on the economy (Sutton et al., 2011). In response to the elevated NO_3^- levels recorded in many water bodies, the European Union (EU) has implemented a number of directives (e.g. Nitrates 91/676/EEC, Water Framework 2000/60/EC, Groundwater 2006/118/EC) that establish a range of measures to reduce NO_3^- contribution from agricultural and non-agricultural sources (Stark and Richards, 2008). Yet controlling groundwater contamination by NO_3^- is still a challenging task, and many EU aquifers have remained heavily contaminated since the introduction of the Nitrates Directive in 1991 (van Grinsven et al., 2012). Reasons for the mixed results are multiple and include the lag-time between measures implementation and improved water quality, the lack of follow through from local actors, but also the complexity of the N cycle and a poor understanding of some *in-situ* mechanisms (Oenema et al., 2011). Additional tools are therefore required to identify the causes of contamination and spatially target existing and future environmental measures. Ideally, accurate NO_3^- source identification is a necessary first step if the contamination problem is to be efficiently addressed. In complex systems however, where several types of NO_3^- sources coexist, source identification and determination of relative contributions become complicated. Such uncertainty is typically witnessed in intensive agricultural areas with high human population densities. In an Irish tillage-dominated catchment historically associated with high NO_3^- occurrence for instance, two studies had attributed the NO_3^- problem to either organic point N sources (stored farmyard wastes and livestock housing, septic tanks) (Daly, 1981) or diffuse applications of artificial N fertilisers (Coxon and Thorn, 1991).

Discriminating between NO_3^- sources such as artificial fertiliser and animal/human organic effluents in rural areas has been central to water studies for decades. Among the methods used to track NO_3^- are dual stable isotope analyses, which measure $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios in dissolved NO_3^- (expressed as $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, respectively). The interest in this technique came from the expectation that some of the major N sources involved in the terrestrial N cycle generate NO_3^- with characteristic and therefore recognizable δ values in groundwater (Kendall et al., 2007). However, NO_3^- is not a conservative ion and once in soil, it is likely to undergo some biochemical reactions and isotopic fractionation that can greatly affect the original δ characteristic values. Heterotrophic denitrification in soil and subsoil is often the cause of a major kinetic isotope effect due to a ^{15}N enrichment factor $\varepsilon_{N2\text{-}NO3}$ (atmospheric dinitrogen N_2 is the reaction product and NO_3^- the substrate) that can vary between -40‰ and -5‰ (Kendall et al., 2007). The consequence of this nitrate removal for the residual NO_3^- pool is that $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ increase linearly over a wide range of values along a slope often reported for groundwater between 0.5 and 0.8 (Granger and Wankel, 2016). This isotopic enrichment in the heavier isotopes can result in NO_3^- from (or derived from) artificial fertiliser displaying $\delta^{15}N\text{-}NO_3^-$ in the range expected for NO_3^- derived from organic wastes (Kendall et al., 2007). The early stage of nitrification in non N-limited systems (e.g. a fertilised field) on the other hand can cause the first newly formed NO_3^- to be quite ^{15}N-depleted compared with the ammonia substrate (NH_3) due to a ^{15}N enrichment factor $\varepsilon_{NO3\text{-}NH3}$ recorded by some between -38‰ and -5‰ (Kendall et al., 2007). But as the N pool is used up, the nitrification rate decreases and so does the isotopic fractionation so that $\delta^{15}N\text{-}NO_3^-$ of newly formed nitrate increases toward the original $\delta^{15}N\text{-}NH_3$ of the substrate over time. The consequence is that leached NO_3^- can display a wide range of $\delta^{15}N\text{-}NO_3^-$ values as nitrification proceeds (Kendall et al., 2007). Finally, mineralisation-immobilisation turnover processes (MIT) (i.e. the rapid remineralisation of NO_3^- assimilated by soil microflora) alter $\delta^{18}O\text{-}NO_3^-$ to within the range expected from nitrification (Mengis et al., 2001) so that synthetic NO_3^- from artificial fertilisers loses its original atmospheric oxygen atoms and therefore its characteristic $\delta^{18}O\text{-}NO_3^-$ signature. As a result, large overlaps may occur between N source types early during the leaching process within the unsaturated zone (Fogg et al., 1998; Minet et al., 2012), hence weakening NO_3^- source tracking in underlying groundwater (Kendall et al., 2007; Xue et al., 2009). More complications can combine within the aquifers under the action of mixing processes between NO_3^- sources, further NO_3^- removal by denitrification (Kendall et al., 2007) and concurrent NO_3^- production during anammox under anoxic conditions (Granger and Wankel, 2016).

To reduce the uncertainties over source identification and fate of NO_3^-, recent research has combined, with a varying degree of success, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ with a number of source apportionment models (Davis et al., 2015; Kim et al., 2015; Xue et al., 2015), groundwater flow dynamics (Hosono et al., 2013), isotopes from non-N species (Stoewer et al., 2015), $\delta^{15}N$ from nitrogenous species other than NO_3^- (Wells et al., 2016), pharmaceutical markers (Fenech et al., 2012) and/or hydrochemical parameters (Pastén-Zapata et al., 2014). The interest in hydrochemistry comes from the fact that besides NO_3^-, many N sources also contain and contribute to the leaching of other ions, which may impact groundwater quality and increase concentrations above natural background levels (NBL) otherwise observed under uncontaminated conditions. Farm animal wastes and septic tank effluents for instance are typically enriched in chloride (Cl^-), potassium (K^+) and sodium (Na^+) amongst many other contaminants, which are all released by decomposing organic matter (Ranjbar and Jalali, 2012). Septic tanks effluents may be further enriched in Na^+ due to human salty diet, the use of water softeners and detergents. Na^+ concentration in farm slurry tanks and septic tank effluents have been commonly measured up to 700 mg L^{-1} (Mulqueen et al., 1999; Sommer and Husted, 1995) and 100 mg L^{-1} (Richards et al., 2016) respectively. Consequently Na^+, which is one of the most mobile of the common cations with low affinity for soil exchange sites (Weil and Brady, 2017), has been used by others as a chemical tracer to delineate plumes of contamination from human organic effluents (Robertson et al., 1991). Yet elevated Na^+ concentration in groundwater used alone can only confirm that contamination by organic wastes occurred. Unlike stable isotopes in NO_3^-, it will not inform on the relative contribution of an organic point source to the NO_3^- issue.

This paper describes a methodology of NO_3^- source identification whereby $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ data were analysed after first classifying groundwater samples according to a combination of contamination indicators. The first indictor was visual and arose from a N source risk assessment survey, with the second indicator being Na^+ concentration in groundwater. This second indicator was used because i) Na^+ is a major component of N-rich organic effluents discharged from septic tanks and farmyards, ii) Na^+ is absent from all main artificial fertilisers applied in the study area, iii) the diffuse application of organic animal wastes was not an important N source and iv) the Na^+ NBL value was low (13 mg L^{-1} in Tedd et al. (2017)). The purpose of this preliminary classification was to apportion groundwater contamination to existing N sources using conventional methods, whereas the superimposition of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ data on this classification was to specifically inform on the contributions of these N sources to NO_3^- contamination and dynamics. Overall, the objective of this study was to explore the applicability of this method for improved investigation of NO_3^- sources and dynamics in the complex environment of the above-mentioned Irish catchment with multiple contamination diffuse and point N sources.

2 MATERIALS AND METHODS

2.1 Study area description

2.1.1 Geology and soil

Groundwater sampling took place in the Barrow Valley (south-east Ireland) across three sites that cover 40 km^2 (Fig.1). The bedrock consists of strata of Carboniferous limestone which underlie Quaternary deposits consisting mainly of fluvioglacial sand and gravel. Quaternary deposit thickness is highly variable but can be up to 25 m, while the individual sand and gravel units, which are usually very coarse, can be up to 10 m thick. Soils are generally well drained. Further details are available in Section 2.1.1 of Materials and Methods SM.

2.1.2 Hydrogeology

There were three main types of aquifers (Fig.1): i) a largely unconfined and highly to extremely vulnerable shallow Quaternary sand and gravel aquifer, which encompasses most sampling wells, ii) a karstic aquifer and iii) a poorly productive bedrock aquifer. Groundwater flow at the regional scale is towards the south and towards the River Barrow (Daly, 1981), although local groundwater pathways can be unpredictable due to the flat topography. Annual rainfall during the sampling period ranged between 606 and 917 mm, whereas annual effective rainfall (i.e. rainfall minus evapotranspiration minus surface runoff), whose daily variations are reported in Fig.2, varied between 179 mm and 464 mm. Groundwater recharge, accounted for by effective rainfall, mostly occurred between October and April of each year, when the soil moisture deficit (SMD) was nil (Fig.2). Air temperature followed a seasonal pattern around its mean (9.6 °C) typical of a temperate maritime climate (Fig.2). A similar pattern was observed with groundwater temperature around 11.5 °C (Fig.2). Further details are available in Section 2.1.2 of Materials and Methods SM.

2.1.3 Land use and management

Soils were primarily dedicated to tillage (Fig.SM-1, adapted from EPA (2017)) for barley and wheat production (64% of land use). Grassland (35% of land use) was used for pasture, silage and hay. Fertilisation largely involved the diffuse application of synthetic non-N (e.g. potash KCl) and N fertilisers, the most popular one by far being Calcium Ammonium Nitrate (CAN). N inputs at the time of the study were routinely excessive for some crops (Teagasc Crop Advisor Officer, personal communication). Due to the predominant tillage land use, the diffuse application of organic animal wastes was not an important N source within the study area. The farm and farmyard density was about 1.7 km^{-2}. Despite few specialising in beef production, many housed some livestock and therefore had some potentially leaking farmyards and manure storage systems. The density of population ranged between 20 and 30 persons km^{-2}. Typically for a rural area, most dwellings were not connected to a public sewerage system, but had their own septic tank instead. Further details are available in Section 2.1.3 of Materials and Methods SM.

2.1.4 Sampling wells

Groundwater samples were collected from 45 wells (Table SM-1). All but one well (B9) were satisfactorily capped and not directly open to air. The type of aquifer intercepted was Quaternary deposits only or Quaternary deposits and bedrock. Further details are available in Section 2.1.4 of Materials and Methods SM.

2.2 Data collection

2.2.1 Groundwater sampling

233 groundwater samples were collected at low and high groundwater recharge times during six sampling campaigns (Fig.2). Each well was generally visited five times or more. Samples were collected directly from the closest tap, which was flamed and cleansed with ethanol, then turned on for up to five minutes to flush the pipe and get water representative of the aquifer. Monitoring

boreholes were purged and sampled with a peristaltic pump. All samples were kept chilled in a cool box during transport to the laboratory. Samples aimed for hydrochemistry analysis and NO_3^- extraction (Section 2.2.2) were filtered on the day of collection with 0.45 μm nylon-membranes before being stored overnight at 4 °C. Further details are available in Section 2.2.1 of Materials and Methods SM.

2.2.2 Groundwater quality and stable isotopes measurements

Groundwater samples were analysed for thirteen physico-chemical and microbial parameters: temperature, conductivity, alkalinity, pH, ion concentrations (NO_3^-, Cl^-, sulphate (SO_4^{2-}), Na^+, K^+, magnesium (Mg^{2+}), calcium (Ca^{2+})), coliforms (total TC and faecal FC). After NO_3^- concentrations were measured, an aliquot of each groundwater sample containing 100 μmol of NO_3^- was portioned off and extracted according to a simplified ion-exchange resin method best suited for freshwater samples with high NO_3^- (> 25 mg L^{-1}) and low dissolved organic carbon (DOC) levels (typically < 5 mg C L^{-1}) (Minet et al., 2011). $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were determined by Continuous-Flow Isotope Ratio Mass Spectrometry in duplicate for each $AgNO_3$ sample encapsulated in silver boats. $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values were expressed in permil (‰) relative to Air and VSMOW respectively, using the standard definition of the δ value of the heavier isotope (h) of a given chemical element (E), $\delta^hE = \{(R_{sample} - R_{std}) / R_{std}\}$, where R represents $^{15}N/^{14}N$ or $^{18}O/^{16}O$ ratios in samples (R_{sample}) and standards (R_{std}) (Kendall et al., 2007). $\delta^{18}O\text{-}H_2O$ and $\delta^2H\text{-}H_2O$ were measured in groundwater samples collected between September 2002 and September 2003 (118/233 samples). Further details are available in Section 2.2.2 of Materials and Methods SM.

2.2.3 N source risk assessment

A N source risk assessment survey was carried out within a 300 m radius around each sampling well. Two main types of N sources reported in Table SM-2 were identified: i) organic point sources, which include septic tanks from unsewered houses and farmyards with potential for N-enriched plumes of contamination and ii) agricultural diffuse sources, which include artificial N fertiliser applications to intensively managed land (tillage and grassland) and soil organic matter (i.e. crop residues, plants, micro- and macro-organisms). Importantly, diffuse sources were ubiquitous as each sampling well was surrounded by more than 75% of intensively managed agricultural land (Table SM-2). % tillage was also estimated because it has long been suspected to be related to diffuse N source contamination in this region (Coxon and Thorn, 1991). Further details are available in Section 2.2.3 of Materials and Methods SM.

2.3 Data analysis

2.3.1 Sample classification and N source apportionment

Groundwater samples were classified according to a two-step methodology (Fig.3). The purpose was to apportion contamination to N source types (Section 3.3.1) using conventional methods before analysing δ^{15}N-NO_3^- and δ^{18}O-NO_3^- (Section 3.3.2). In Step 1, groundwater samples were classified based on the presence/absence of organic point sources within a 300 m radius of sampling wells using N source risk assessment data (Table SM-2). To increase the power of the classification, it was decided to add the second indicator. In Step 2, groundwater was classified based on the exceedance/non-exceedance of a Na^+ contamination threshold (CT), Na^+ being a major component of N-rich organic effluents discharged from septic tanks and farmyards. Any exceedance of the CT value was deemed indicative of point source contamination because Na^+ is not a major constituent of the main artificial fertilisers applied in the study area (unlike other indicators like Cl^- and K^+) and animal waste was not an important diffuse N source (Section 2.1.3). This CT value was not strictly speaking a NBL but it gave a picture of the Na^+ concentration in the less contaminated groundwater. It was calculated as the 75th percentile of Na^+ concentration from twenty-nine low NO_3^- samples (< 25 mg L^{-1}) from the study area (Section 3.3.1) in order to exclude outliers from groundwater highly contaminated by a point source but severely denitrified. The main limitation of Step 2 came from the fact that Na^+ is not as mobile in soils as NO_3^-, but it is nonetheless one of the most mobile of the common cations whose adsorption should be further reduced as the sorption sites within plumes of contamination become saturated.

Where both steps of the classification agreed over the impact (or the lack of impact) of point sources, the origin of contamination was deemed beyond reasonable doubt and samples could be categorised into two reference groups (Fig.3): agricultural diffuse source contamination only (label D+P-) or agricultural diffuse and organic point source contamination (label D+P+). In the case of D+P- samples, the lack of evidence for point source contamination directed by default towards diffuse sources alone (artificial fertiliser and/or soil organic matter), i.e. the only N source identified in the survey around the wells that could explain the high NO_3^- concentrations. In the case of D+P+ samples, the impact of point source contamination was characterised, but its NO_3^- contribution relative to the ubiquitous diffuse sources (whose impact was assumed) was unknown. When both steps disagreed over the influence of point sources, samples were classified as D+P± (i.e. impact of point source ambiguous).

It should be noted that this classification (and Step 2 in particular), did not discriminate between septic tank and farmyard effluent contaminations, a topic of great interest in some of the literature. Such differentiation would have been vain since every sampling well with a farmyard within a 300 m radius also had the septic tank from the farm house within that radius.

2.3.2 Statistical analysis

The relationships between hydrochemical parameters and other variables (number of point sources in Table SM-2) were investigated by Spearman's rank order correlation coefficients (r_s) and significance levels. To compare means between categories of independent variables (% tillage, N source apportionment), dependent variables (NO_3^- concentration, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$) were analysed in the following mixed factorial model: time × either % tillage (groups 0-50% and 50-100%) or N source apportionment classification (groups D+P+, D+P- and D+P± as in Fig.3), with site (C, B and O) as a random blocking effect. There were repeated measurements made on the experimental units (sampling wells) and the correlation over time was taken into account using an unstructured covariance model. Pairwise comparisons were made with Tukey-Kramer posthoc tests. The model was fitted in the Mixed procedure of SAS 9.4 (2014), and all the data were log transformed because of non-constant variance.

3 RESULTS AND DISCUSSION

3.1 Probing NO_3^- source identification with groundwater quality and N source risk assessment

3.1.1 Introduction to groundwater quality and the NO_3^- contamination problem

NO_3^- concentration in groundwater samples ranged between 2.0 and 134.9 mg L^{-1} NO_3^- (median of 47.9 mg L^{-1}) with few low nitrate concentration samples (Fig.4). The severity of the NO_3^- problem in the area was highlighted as 88% and 45% of the samples had NO_3^- concentration exceeding 25 and 50 mg L^{-1}, respectively. (Note that 50 mg L^{-1} NO_3^- is both the EU drinking water limit for NO_3^- and also the NO_3^- standard for good status of groundwater bodies under the EU Groundwater Directive). At each well, NO_3^- concentration fluctuated between sampling events (median range of 17.5 mg L^{-1}). Only four wells consistently had low NO_3^- concentrations (< 25 mg L^{-1}), whereas thirteen wells had consistently high NO_3^- concentrations (> 50 mg L^{-1}).

Unlike NO_3^-, other physico-chemical parameters complied with their respective Drinking Water Directive limit, set at 200 mg L^{-1} for Na^+, 250 mg L^{-1} for Cl^- and SO_4^{2-}, between 6.0 and 9.5 for pH, 2500 µS cm^{-1} for conductivity. However, these limits are elevated and may not be relevant to detect pollution problems. Far more informative are the NBLs measured under uncontaminated conditions in similar aquifers as defined in Tedd et al. (2017) for Irish aquifers. In that respect, parameters associated with contamination from both N/non-N artificial fertiliser and animal/human waste effluents (K^+ and Cl^-) or animal/human wastes only (Na^+ and FC) displayed some values widely in exceedance of such NBLs (Fig.4). Na^+ varied between 4 and 46 mg L^{-1} (median of 11), and 32% of the samples exceeded the NBL reported at 13 mg L^{-1} in Tedd et al. (2017). K^+ varied between 0.2 and 63 mg L^{-1} (median of 1.9), and 48% of the samples exceeded the NBL reported at 2.1 mg L^{-1}. As for Cl^-, it varied between 5.1 to 82.9 mg L^{-1} (median of 29.6), and 85% of the samples exceeded the NBL reported at 21 mg L^{-1}. Other parameters were equally variable: SO_4^{2-} varied between 8.8 and 68.8 mg L^{-1} (median of 31.2) and Mg^{2+} varied between 2.2 and 28.0 mg L^{-1} (median of 14.0). From the bacterial perspective, 70.2% of samples tested positive to TC, whereas 19.9% tested positive to FC. Samples positive to FC originated from fifteen wells that displayed presence of FC on at least one occasion. The exceedance of NBL values and the presence of FC reflect the detrimental influence of anthropogenic activities (highly managed agricultural land and/or discharge of organic animal/human wastes effluents from farmyards/septic tanks) on groundwater quality. Other parameters such as calcium (Ca^{2+}) concentration (88 to 190 mg L^{-1}, median of 122), alkalinity (210 to 526 mg $CaCO_3$ L^{-1}, median of 308), pH (6.8 to 7.6, median of 7.3) and conductivity (584 to 1206 µS cm^{-1}, median of 784) generally reflect hydrogeological conditions and limestone buffering.

In line with Fig.2, Fig.5 highlights that groundwater originated mainly from autumn and winter precipitation, a period particularly conducive of NO_3^- leaching from agricultural soils (Premrov et al., 2012). The isotopic composition of groundwater samples ($\delta^{18}O-H_2O$ between -8.9‰ and -6.5‰, $\delta^{2}H-H_2O$ between -51.8‰ and -36.1‰), collected 170 km from the Global Network for Isotopes in Precipitation (GNIP) station of Valentia Island (southwest Ireland), plotted slightly above the Local meteoric Water Line (LMWL) drawn from the GNIP station data (IAEA, 2017). It was most similar with winter and autumn precipitation (mean $\delta^{18}O-H_2O$ of -6.4‰ and -6.0‰ in Fig.5, respectively). In comparison, spring and summer precipitation (mean $\delta^{18}O-H_2O$ of -4.9‰ and -4.1‰, respectively) exhibited higher values. Precipitation typically displays $\delta^{2}H-H_2O$ and $\delta^{18}O-H_2O$ values lower in winter than in summer, which is mainly controlled by the amount of water rained-out. As water vapour derived from the evaporation of low-latitude oceans moves northwards with air masses, winter precipitation, which is more intense than summer one because of colder condensation temperature, discharges larger amounts of ^{2}H- and ^{18}O-enriched rainfall than during summer (Mook and de Vries, 2000). Consequently, remaining water vapour that reaches temperate countries like Ireland shows lower δ^2H-H_2O and $\delta^{18}O$-H_2O values in winter, which generates lower δ^2H-H_2O and $\delta^{18}O$-H_2O values in winter precipitation (in comparison with summer rainfall). In addition to the seasonal effect, δ^2H-H_2O and $\delta^{18}O$-H_2O values in precipitation are also subjected to several other effects causing regional and temporal variations such as the latitudinal effect (lower δ values at increasing latitude), the continental effect (more negative δ values the more inland), the altitude effect (decreasing δ values at higher altitude) and the amount effect (lower δ values during heavy storms) (Mook and de Vries, 2000).

3.1.2 Relationships between hydrochemistry and N source risk assessment: information gained and limitations

There were several significant yet ambiguous relationships between groundwater NO_3^- concentration and chemical indicators relevant to both diffuse and point source contamination (Table 1). NO_3^- concentration was positively yet weakly correlated with Na^+ concentration ($r_s = 0.133$, $p < 0.05$), which could be due to the small impact of point sources (i.e. animal/human wastes effluents) on NO_3^- groundwater loading. NO_3^- had a stronger positive correlation with Cl^- ($r_s = 0.342$, $p < 0.0001$), but this could be indicative of contamination from diffuse applications of KCl (Section 2.1.3) and/or organic point sources. No significant relationship was detected with K^+ (contained in both N-K-P fertilisers and animal/human wastes), which is generally strongly held by soil particles (Weil and Brady, 2017). NO_3^- was positively correlated with Ca^{2+} ($r_s = 0.479$, $p < 0.0001$), which is a major component of both the widely used CAN fertiliser and subsoil/bedrock materials. NO_3^- was also significantly but weakly correlated with other parameters such as SO_4^{2-} and alkalinity, whereas stronger significant relationships were observed with Mg^{2+} ($r_s = -0.301$, $p < 0.0001$) and conductivity ($r_s = 0.472$, $p < 0.0001$). The pH was negatively correlated with a number of parameters, but not with NO_3^-, which could have otherwise been indicative of denitrification (Rivett et al., 2008). Interestingly though, pH was strongly and negatively correlated with $\delta^{15}N$-NO_3^- ($r_s = -0.513$, $p < 0.0001$) and $\delta^{18}O$-

NO_3^- ($r_s = -0.401$, $p < 0.0001$), which is discussed in Section 3.2.1. Many other relationships were weak ($r_s < 0.3$) and/or not significant ($p > 0.05$) (Table 1).

Negative correlations were observed between NO_3^- concentration and the number of unsewered houses ($r_s = -0.189$, $p < 0.01$) and farmyards ($r_s = -0.181$, $p < 0.01$). These relationships were unexpected because a higher loading of organic waste effluents is assumed to increase NO_3^- concentration. However, these correlations were also weak, which could simply indicate that point sources are not major NO_3^- sources. Alternatively, the lowering of NO_3^- concentration might be due to denitrification enhanced by higher DOC levels (Jahangir et al., 2014) and sustained by a higher density of point sources. Nonetheless, all wells with more than ten unsewered houses within 300 m radius were located in site O, where the % tillage was lower and % grassland was higher (Table SM-2). Therefore, the negative correlation might be caused by land use and reduced NO_3^- leaching at site O.

Other relationships in Table 1 are of particular interest. Most prominently the concentration of Na^+, which is used as an indicator of point source contamination in Section 3.3.1, was positively correlated with the number of unsewered houses ($r_s = 0.317$, $p < 0.0001$) and farmyards ($r_s = 0.247$, $p < 0.001$). A closer examination of this relationship in Fig.6 reveals that in the absence of any point source within a 300 m radius (i.e. no unsewered house, which also meant no farm house and its farmyard), Na^+ concentration showed little variation and remained low below 9 mg L^{-1}, i.e. below the NBL of 13 mg L^{-1} (Tedd et al., 2017). These samples with no nearby point sources were all collected from monitoring piezometers with 75-100% tillage within a 300 m radius. This ruled out high % tillage and the decomposition of crop residues as an important source of Na^+ leaching. It should be noted that the correlation coefficient r_s between Na^+ concentration and the number of houses further increased from 0.317 to 0.545 when keeping % tillage constant at 75-100%, i.e. the largest % tillage group by far (65% of the samples). Added to the presence of FC in some samples (Section 3.1.1) and the fact that Na^+ is a major component of organic point source effluents, these results confirm that animal/human waste effluent discharges had some impact on groundwater quality. Yet these results did not inform about the NO_3^- contamination source and the importance of point sources relative to the diffuse source pollution.

From the landuse perspective, NO_3^- concentration was significantly higher ($p < 0.0001$) in samples collected from wells with more than 50% tillage within a 300 m radius (mean of 53.3 mg L^{-1}) than if % tillage was below 50% (mean of 33.7 mg L^{-1}). This is consistent with NO_3^- contamination from an agricultural diffuse N sources, which could be the diffuse applications of artificial N fertiliser and/or the mineralisation and nitrification of soil organic N enhanced by soil aeration post ploughing and fertilisation.

3.2 Investigating NO_3^- sources and dynamics using dual stable isotope analyses alone

Except for two outliers slightly above 18‰, $\delta^{15}N\text{-}NO_3^-$ in groundwater ranged between 2.2‰ and 12.1‰ (median of 5.5‰), while $\delta^{18}O\text{-}NO_3^-$ ranged between -2.4‰ and 10.9‰ (median of 2.2‰) (Fig.7). δ values in individual wells were quite stable over time. With disregard for the two outliers, the temporal difference between minimum and maximum $\delta^{15}N\text{-}NO_3^-$ values was smaller than 5‰ in 43 wells (< 2‰ in 32 wells). Similarly, the variation in $\delta^{18}O\text{-}NO_3^-$ was below 5‰ in 42 wells (< 3‰ in 28 wells). Overall, both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ variables were positively and significantly correlated ($r_s = 0.599$, $p < 0.0001$) along a linear regression line with a slope of 0.50 (result not shown).

3.2.1 Biochemical and mixing processes

A highly significant correlation between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ ($r_s = 0.599$, $p < 0.0001$) with a positive slope of 0.504 was indicative of denitrification (Granger and Wankel, 2016; Kendall et al., 2007), which had been anticipated to be more limited due to the elevated levels of dissolved oxygen (O_2) measured between 9 and 11 mg L^{-1} in the sand and gravel deposits of site O (Premrov et al., 2012) and despite the presence of clay lenses. The strong negative correlation ($r_s = -0.513$, $p < 0.0001$)

between $\delta^{15}N\text{-}NO_3^-$ and groundwater pH (Table 1) may be seen as a further evidence of heterotrophic denitrification as the microbial reduction of NO_3^- may induce an increase in pH (Rivett et al., 2008).

The plotting of $\delta^{15}N\text{-}NO_3^-$ against $1/NO_3^-$ concentration should in theory yield a straight line if the mixing of two groundwater endmembers with distinct NO_3^- concentrations and isotopic δ values occurs (Kendall et al., 2007). However no such relationship was observed (result not shown). At the scale of the study area, this result provides no evidence for contamination by two N sources although at a smaller scale, closer to point sources, the characteristic straight line might have been observed. The characterisation of mixing processes may yet be complicated by the occurrence of denitrification and the fact that the isotopic composition of end-members rarely stays constant over time (Kendall et al., 2007).

3.2.2 NO_3^- sources

NO_3^- source identification based on stable isotopes is carried out by comparing $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ measured in groundwater with $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ expected from various N sources. The expected $\delta^{15}N\text{-}NO_3^-$ values derive directly from $\delta^{15}N\text{-}TN$ (i.e. $\delta^{15}N$ in total nitrogen) measured in N source materials. The mineralisation of TN into ammonium (NH_4^+) causes little isotopic fractionation and $\delta^{15}N\text{-}NO_3^-$ shifts towards $\delta^{15}N\text{-}TN$ of the source as nitrification goes on in non N-limited systems (Kendall et al., 2007), so N sources are expected to generate NO_3^- with $\delta^{15}N\text{-}NO_3^-$ similar to the original $\delta^{15}N\text{-}TN$ values in the absence of any interfering process like denitrification. Due to its atmospheric origin, $\delta^{15}N\text{-}TN$ in artificial fertilisers often varies between -4‰ and 4‰ (Wassenaar, 1995), which contrasts with the distinctly higher $\delta^{15}N\text{-}TN$ values in animal/human organic wastes that has generally been reported to generate $\delta^{15}N\text{-}NO_3^-$ between 10‰ and 20‰ due to enhanced NH_3 volatilisation (Macko and Ostrom, 1994). Between these two sources, $\delta^{15}N\text{-}TN$ in soils (mostly organic N) frequently varies between 4‰ and 9‰ (Heaton, 1986), although wider ranges are sometimes reported (Kendall et al., 2007). In parallel to $\delta^{15}N$, $\delta^{18}O\text{-}NO_3^-$ in artificial fertilisers nitrate often ranges between 18‰ and 22‰ (Amberger and Schmidt, 1987), whereas $\delta^{18}O\text{-}NO_3^-$ arising from nitrification is much lower and may be expected between 0‰ and 5‰ (Durka et al., 1994), although wider ranges are sometimes reported (Kendall et al., 2007). These $\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$ values expected for the different types of N sources and commonly used in the literature were reported in Fig.7 for comparison with δ values measured in groundwater NO_3^-. It is noteworthy that apart from septic tank effluents, which had not been directly sampled in this study, artificial fertiliser commonly used in the local area ($\delta^{15}N$-TN and $\delta^{18}O-NO_3^-$), dairy slurry ($\delta^{15}N$-TN) and local agricultural soil ($\delta^{15}N$-TN) samples analysed from agricultural research sites for a related soil zone study (Minet et al., 2012) displayed δ values within the above-mentioned ranges.

The observed groundwater $\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$ values in Fig.7 overlapped with the expected ranges from three N sources: nitrified artificial fertiliser N, soil organic N and animal/human waste N. However, data interpretation should take into account the occurrence of the overall denitrification line, which starts somewhere in the nitrification zone between $\delta^{15}N-NO_3^-$ of 2‰ and 6‰, i.e. between the high end of the $\delta^{15}N-NO_3^-$ range expected for nitrified artificial fertiliser N and the low end of the $\delta^{15}N-NO_3^-$ range expected for soil organic N-derived NO_3^-. This 2-6‰ range could be indicative of the nitrification of soil organic matter that had incorporated ^{15}N-depleted nitrogen from artificial fertiliser applications (Choi et al., 2017) and is later leached during groundwater recharge. This interpretation of a single NO_3^- source contamination process is consistent with studies that designate soil organic matter as an important source of NO_3^- leaching in intensively managed grassland (Minet et al., 2012) or in tillage (Savard et al., 2010). On the other hand, the direct leaching of artificial NO_3^- (contained in applied artificial fertiliser) and nitrified artificial fertiliser N (e.g. NO_3^- derived from NH_4^+ in CAN) might not be an immediate source of contamination. The significantly higher NO_3^- concentration where % tillage within a 300 m radius was above 50 (Section 3.1.2) was not accompanied by a significant lower $\delta^{15}N-NO_3^-$ ($p > 0.05$), which could otherwise be observed with the direct leaching of fertiliser-derived NO_3^- (i.e. artificial NO_3^- and nitrified fertiliser N) through the unsaturated zone (Minet et al., 2012). This should not come as a surprise considering that most fertiliser N applied to soil and not already taken up by plants is rapidly immobilised by soil micro-organisms and therefore, little is left unused and available for leaching (Powlson et al., 1992). Had the base of the denitrification line been broad enough to encompass the range of δ values expected from animal/human waste point sources, more than one contamination source would have been considered (Clague et al., 2015). Nevertheless, the strong positive correlations between $\delta^{15}N-NO_3^-$ and concentrations of other indicators such as Na^+ ($r_s = 0.488$, $p < 0.0001$), Cl^- ($r_s = 0.363$, $p < 0.0001$) and K^+ ($r_s = 0.370$, $p < 0.0001$) in Table 1 may still suggest that organic point sources play some role in the contamination process, which is further discussed in Section 3.3. In comparison, there were few strong and significant relationships between $\delta^{18}O-NO_3^-$ and chemical parameters.

The ability of soils to recycle N through MIT processes probably explains why no sample displayed elevated $\delta^{18}O-NO_3^-$ values associated with artificial NO_3^- (Mengis et al., 2001). In addition, N in the applied fertilisers was partly in an artificial non-NO_3^- form so that the expected $\delta^{18}O-NO_3^-$ range for the entire pool of fertiliser-derived NO_3^- is lower than the 18-22‰ range for artificial NO_3^- (Minet et al., 2012). Most $\delta^{18}O-NO_3^-$ values were in fact within the 0-5‰ range expected from nitrification by Durka et al. (1994). This 0-5‰ range was calculated under the debatable assumption (Minet et al., 2012) that the oxidation of NH_3 into nitrite (NO_2^-) incorporates without isotopic fractionation one oxygen from diatomic O_2 and one oxygen from H_2O followed by the final incorporation of one oxygen from H_2O during NO_2^- oxidation. If using local $\delta^{18}O-H_2O$ values (between -8.9 and -6.5‰ in Fig.5) and $\delta^{18}O-O_2$ of 23.5‰ (Kroopnick and Craig, 1972), this calculated nitrification range can be further narrowed to 1.9-3.5‰. It encompasses only 36% of $\delta^{18}O-NO_3^-$ values whereas a majority of samples (41%) displayed values that were lower (minimum of -2.4‰). Recent research now demonstrates that various kinetic isotopic fractionation factors operate during oxygen incorporations and NO_2^- oxidation (Granger et al., 2016) and therefore, the calculated nitrification range of 1.9-3.5‰ is most likely biased. Importantly, $\delta^{18}O-NO_3^-$ of newly formed nitrate in soils or aquatic systems can be deeply affected by a $H_2O-NO_2^-$ oxygen exchange and the associated equilibrium isotopic fractionation (Granger and Wankel, 2016). The extent of this oxygen exchange is extremely variable and it may be driven by the duration of NO_2^- accumulation, the longer the duration the larger the exchange with incidentally the loss of some oxygen of diatomic O_2 origin (Buchwald et al., 2012). The absence of $\delta^{18}O-NO_3^-$ values close to those of $\delta^{18}O-H_2O$ values suggests that no large oxygen exchanges occurred in our study. This is consistent with the absence of NO_2^- detected in all the groundwater samples (Materials and Methods SM), including those where elevated levels could have been expected (contamination group D+P+ from Fig.3).

3.3 Improving data interpretation by combining dual stable isotopes with indicators of point source contamination

3.3.1 N source apportionment using indicators of point source contamination

A Na^+ CT value was calculated (based on Section 2.3.1) at 11.4 mg L^{-1}, i.e. below the 13 mg L^{-1} NBL defined in Tedd et al. (2017). As anticipated, the '75th percentile' calculation excluded low NO_3^- samples with strong indications of contamination (Cl^- concentration between 25.6 and 63 mg L^{-1}) but signs of denitrification (elevated $\delta^{15}N\text{-}NO_3^-$ between 7.8 and 18.4‰). Groundwater samples could then be apportioned to N source types as per the methodology in Fig.3. As a result, 22 samples categorised D+P- displayed Na^+ concentrations within a 4.0-9.0 mg L^{-1} range (Table SM-3). They were associated with contamination from agricultural diffuse N sources only (i.e. artificial fertiliser N and/or soil organic matter). On the other hand, 99 samples categorised D+P+ displayed Na^+ concentrations within a 11.6-46.0 mg L^{-1} range (Table SM-3). They were associated with diffuse and also point N sources (i.e. septic tanks from unsewered houses and/or farmyards). For the remaining 112 samples labelled D+P±, evidence of point source contamination was contradictory ('Yes' for Step1 always followed by 'No' for Step2 in Fig.3) but Na^+ concentration was within a 6.9-11.3 mg L^{-1} range (Table SM-3), i.e. below the Na^+ CT value of 11.4 mg L^{-1}. These uncertainties in group D+P± possibly relate to sampling well locations (point source plumes not being intercepted), some Na^+ adsorption in soil or simply low effluent discharge. Tellingly, no D+P± sample was collected from a well without a nearby point source but with a Na^+ concentration above the CT value ('No' for Step1 was never followed by 'Yes' for Step2 in Fig.3), which appears to confirm the validity of the two step approach as this combination should not be observed. Samples collected from most wells (thirty-one out of forty-four wells sampled more than once) consistently belonged to the same category, which could be expected should one type of source dominate (although seasonal changes in hydrogeological conditions might affect contamination processes and cause wells to switch category). It can be noted that unlike Na^+ concentration, other measured parameters widely overlapped between D+P+ and D+P- categories (Table SM-3), which highlights that they were probably not appropriate indicators of one type of N source contamination in the context of the study area.

The contrast created by the sample classification between D+P- and D+P+ categories was useful to characterise the occurrence of point source contamination in an intensively managed agricultural area. However, it did not inform on the relative contributions of agricultural diffuse and organic point sources to the NO_3^- issue. Although the measurement of an elevated Na^+ contamination in the vicinity of septic tanks and farmyards is most likely an evidence of point source contamination, it does not necessarily mean that the point source NO_3^- input to groundwater outweighs the diffuse input from fertiliser- and/or soil organic matter-derived NO_3^- leached below agricultural soils. The analysis of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ in Section 3.3.1 aimed to resolve this uncertainty.

3.3.2 Fine-tuning results interpretation

The scatterplot of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ superimposed on the N source apportionment classification for groundwater samples is presented in Fig.7. The effect of classification on $\delta^{15}N\text{-}NO_3^-$ was significant ($p < 0.0001$), with all pairwise comparisons between groups also significant. D+P- values (mean $\delta^{15}N\text{-}NO_3^-$ of 4.4‰) were significantly lower ($p < 0.0001$) than D+P+ values (mean $\delta^{15}N\text{-}NO_3^-$ of 7.1‰). D+P± values were intermediate (mean $\delta^{15}N\text{-}NO_3^-$ of 5.2‰), significantly higher than D+P- values ($p = 0.005$) and significantly lower than D+P+ values ($p < 0.0001$). The reason for these differences between groups may relate to the differential occurrence of denitrification, as significant positive relationships between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were detected in groups D+P+ ($r_s = 0.650$, $p < 0.0001$) and D+P± ($r_s = 0.618$, $p < 0.0001$), but not in group D+P- (Fig.7). The fact that $\delta^{15}N\text{-}NO_3^-$ values in group D+P+ were higher than in group D+P± suggests a more advanced denitrification (i.e. less NO_3^- left in groundwater) where point source contamination was clearly characterised. In comparison with $\delta^{15}N\text{-}NO_3^-$, no significant classification effect ($p > 0.05$) was detected for $\delta^{18}O\text{-}NO_3^-$, possibly because $\delta^{18}O\text{-}NO_3^-$ is less affected by denitrification than $\delta^{15}N\text{-}NO_3^-$.

The denitrification lines for the D+P+ and D+P± groups started in the same $\delta^{15}N-NO_3^-$ zone of 2-6‰ (Fig.7) as all but one D+P- sample (sample with $\delta^{15}N-NO_3^-$ of 18.6‰), a range that could be related to nitrification from soil organic matter that has incorporated ^{15}N-depleted nitrogen from artificial fertilisers (Section 3.2.2). This interpretation with well defined denitrification lines that have the same origin ruled out the discharge of animal/human waste effluents from point sources as a main contributor to groundwater NO_3^- contamination, which should have otherwise broaden the distribution of $\delta^{15}N-NO_3^-$ towards higher values making the characterisation of denitrification possibly more difficult (Clague et al., 2015). Instead, we hypothesise that point source discharge increased the levels of DOC in the vicinity of wells, which promoted reducing conditions for NO_3^- and therefore denitrification observed with most samples (groups D+P+ and D+P ± represented 211/233 samples, and 40/45 sampling wells). The seemingly lesser denitrification affecting D+P± samples may just reflect a more limited impact of point sources at these sampling wells. The absence of a positive relationship observed between $\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$ with samples D+P- confirmed the original hypothesis that despite the presence of clay lenses, the free-draining nature of the subsoil combined with the high O_2 level and low DOC (Premrov et al., 2012) limited the potential for denitrification. The examination of Fig.7 and group D+P- in particular also suggests that some synthetic fertiliser NO_3^- may have leached down to groundwater as some D+P- samples with low $\delta^{15}N-NO_3^-$ value (3.4-3.9‰) exhibited $\delta^{18}O-NO_3^-$ distinctly higher (6.6-8.0‰). However, this was unusual and the direct leaching of artificial fertiliser NO_3^- was not widespread in the study area, despite conditions that might have favoured it (shallow water-table overlain by free-draining soil, elevated precipitation, high fertiliser application rates). Similar results, also observed in the unsaturated zone (Minet et al., 2012), were not surprising since soil microbes usually leave very little nitrate unused in soil following application (Section 3.2.2).

In the context of the study area, dual stable isotopes used alone (Section 3.2.2) helped identify the most likely main contributor to groundwater NO_3^- contamination (nitrification of soil organic matter as an agricultural diffuse source, which is stimulated by fertiliser application and tillage) as well as characterise denitrification. The combined use of two contamination indicators (as described in Fig.3) with δ values helped identify conditions for denitrification to take place, i.e. the presence of organic point sources near sampling wells. In a more complex environment, this methodology and the use of additional indicators of contamination, e.g. pharmaceutical molecules specific to animals or to humans (Fenech et al., 2012) added to Fig.3 classification, could help further categorise groundwater samples and differentiate between NO_3^- contamination from septic tanks and from farmyard sources, which could not be achieved with Na^+ concentration.

4 CONCLUSIONS

- Plotting $\delta^{15}N\text{-}NO_3^-$ against $\delta^{18}O\text{-}NO_3^-$ revealed a strong significant correlation between both variables ($p < 0.0001$, $r_s = 0.599$, slope of 0.5), which was indicative of denitrification. After comparison with the δ values expected from various N sources, the origin of the regression line suggested that groundwater contamination by NO_3^- was dominated by an agricultural diffuse N source (most likely the large organic matter pool that has incorporated ^{15}N-depleted nitrogen from artificial fertiliser in agricultural soils).
- Two indicators of organic point source contamination, presence/absence of organic point sources (i.e. septic tank and/or farmyard) near sampling wells and exceedance/non-exceedance of a contamination threshold value for Na^+ in groundwater, showed their relevance to characterise the influence of organic point sources on groundwater quality in many groundwater samples.
- The classification of groundwater samples according to the combination of both indicators followed by the analysis of the scatterplot of $\delta^{15}N\text{-}NO_3^-$ against $\delta^{18}O\text{-}NO_3^-$ showed that denitrification occurred only in samples where point source contamination was characterised or suspected, possibly because of a high DOC concentration in groundwater.
- Combining contamination indicators and a large stable isotopes dataset collected over a large study area helped i) to identify the main source of groundwater NO_3^- and ii) to associate the occurrence/non-occurrence of a microbial process (denitrification) to one type of contamination

(organic point source), which improved our understanding of NO_3^- contamination processes in groundwater.

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Figure 1: Study area with sampling well locations (n = 45), 10 m elevation contour lines (m above sea level), gravel and bedrock aquifer boundaries (adapted from GSI (2017), with 'Extended gravel' area referring to fluvioglacial sand and gravel with thickness ≥ 9 m at wells C5 and C10 and therefore suggested as part of the gravel aquifer).

Figure 2: Daily effective rainfall (mm), soil moisture deficit or SMD (mm), mean daily air temperature (°C) and mean groundwater temperature (mean for each sampling campaign ± standard deviation). (Shaded boxes on the time axis refer to the different sampling campaigns.)

Figure 3: Framework to classify groundwater samples and apportion contamination to the following types of N sources: D+P+ (Diffuse and Point), D+P- (Diffuse only) and D+P± (Diffuse, Point ambiguous). (CT stands for Na^+ contamination threshold, as defined in Section 2.3.1.; averages and ranges of values for each measured physico-chemical parameter within each contamination group are presented in Table SM-3.)

Figure 4: Boxplots of ion concentrations (NO_3^-, Na^+, K^+, Cl^-, SO_4^{2-}, Mg^{2+} and Ca^{2+} in mg L^{-1}), pH (pH units), alkalinity (mg $CaCO_3$ L^{-1}) and conductivity (µS cm^{-1}). (Boxes represent the central half of the data, with the bar in the middle as the median; start of left whisker and end of the right

whisker represents the lowest and highest values that are not outliers; circles are outliers outside 10th and 90th percentiles.)

Figure 5: Scatterplot of $\delta^{18}O\text{-}H_2O$ and $\delta^2H\text{-}H_2O$ (‰ VSMOW) in groundwater samples collected in the Barrow Valley study area between September 2002 and 2003 (n = 118) and precipitation collected at the Irish GNIP station of Valentia Island between January 2000 and September 2003 (n = 45) (astronomical seasons based on spring and autumnal equinoxes, winter and summer solstices). (The solid Ordinary Least Square regression line (Hughes and Crawford, 2012), with Spearman Rank correlation coefficient and p value, represents the meteoric water line for Valentia Island precipitation.)

Figure 6: Scatterplot of Na^+ concentration and the number of unsewered houses within a 300 m radius (samples from well B6 and C10 excluded because of communal septic tanks, n = 222).

Figure 7: Scatterplot of $\delta^{15}N\text{-}NO_3^-$ (‰ Air) and $\delta^{18}O\text{-}NO_3^-$ (‰ VSMOW) in groundwater samples across the following categories of N source contamination: D+P+ (Diffuse and Point, n = 99), D+P- (Diffuse only, n = 22) and D+P± (Diffuse but point uncertain, n = 112). (Regression lines apply only to D+P+ and D+P± groups where relationships were significant; arrows describing microbial processes and boxes that delineate commonly expected δ values for NO_3^- derived from several N sources present in the study area are reported in the following references: Wassenaar (1995) for artificial fertiliser $\delta^{15}N$, Heaton (1986) for soil $\delta^{15}N$, Macko and Ostrom (1994) for animal/human waste $\delta^{15}N$, Amberger and Schmidt (1987) for artificial fertiliser $\delta^{18}O$, Durka et al. (1994) for nitrification $\delta^{18}O$, Mengis et al. (2001) for MIT processes, Granger and Wankel (2016) for denitrification.)

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, $p \leq 0.001$ ***, $p \leq 0.0001$ **; correlations with $p > 0.05$ not reported; relationships with $r_s > 0.3$ are shaded.)

LIST OF SUPPLEMENTARY MATERIALS

Figure SM-1: Land use in the Barrow Valley sampling area in 2000, with boundaries of the Electoral Divisions (adapted from EPA (2017)).

Table SM-1: Description of the sampling wells (ND stands for Not Determined).

Table SM-2: Point N sources (farmyards, houses) and diffuse N sources (includes % agricultural land and % tillage) present within 300 m radius of sampling wells.

Table SM-3: Averages and ranges of measured physico-chemical parameters within the following contamination groups: D+P+ (Diffuse and Point, n = 99), D+P- (Diffuse only, n = 22) and D+P± (Diffuse but point uncertain, n = 112).

Materials and Methods SM

Table 1

Table 1: Correlation matrix of Spearman's rank correlation coefficients r_s between hydrochemical and stable isotopes (in NO_3^-) variables and the number of point sources (unsewered houses, farmyards) within 300 m radius around sampling wells. (Significance levels: $p \leq 0.05$ *, $p \leq 0.01$ **, $p \leq 0.001$ ***, $p \leq 0.0001$ ****; correlations with $p > 0.05$ not reported; strongest relationships with $r_s > 0.3$ are shaded.)

Parameter	**NO_3^-**	**Cl^-**	**K^+**	**Na^+**	**SO_4^{2-}**	**Mg^{2+}**	**Ca^{2+}**	**pH**	**Conductivity**	**Alkalinity**	**$\delta^{15}N$-NO_3^-**	**$\delta^{18}O$-NO_3^-**
Cl^-	0.342****											
K^+	-	0.251***										
Na^+	0.133*	0.715****	0.366****									
SO_4^{2-}	0.256****	0.191**	0.180**	0.259****								
Mg^{2+}	-0.301****	-	-0.168*	-								
Ca^{2+}	0.479****	0.293****	0.304****	0.256****	0.242***	-0.331****						
pH	-	-0.203**	-0.359****	-0.252***	-	-	-0.417****					
Conductivity	0.472****	0.581****	0.413****	0.539****	0.442****	-	0.787****	-0.504****				
Alkalinity	-0.179**	-	0.386****	0.139*	-	0.183**	0.347****	-0.629****	0.471****			
$\delta^{15}N$-NO_3^-	-	0.363****	0.370****	0.488****	0.335****	-	0.321****	-0.513****	0.544****	0.493****		
$\delta^{18}O$-NO_3^-	-0.281****	-	-	0.159*	0.141*	-	-	-0.401****	0.231***	0.453****	0.599****	
Houses[a]	-0.189**	0.146*	-	0.317****	-	0.192**	-	-	-	-	0.168*	-
Farmyards	-0.181**	0.298****	-	0.247***	-	0.331****	-	-	0.225**	0.182**	0.457****	0.226**

Figure 1

Figure 1

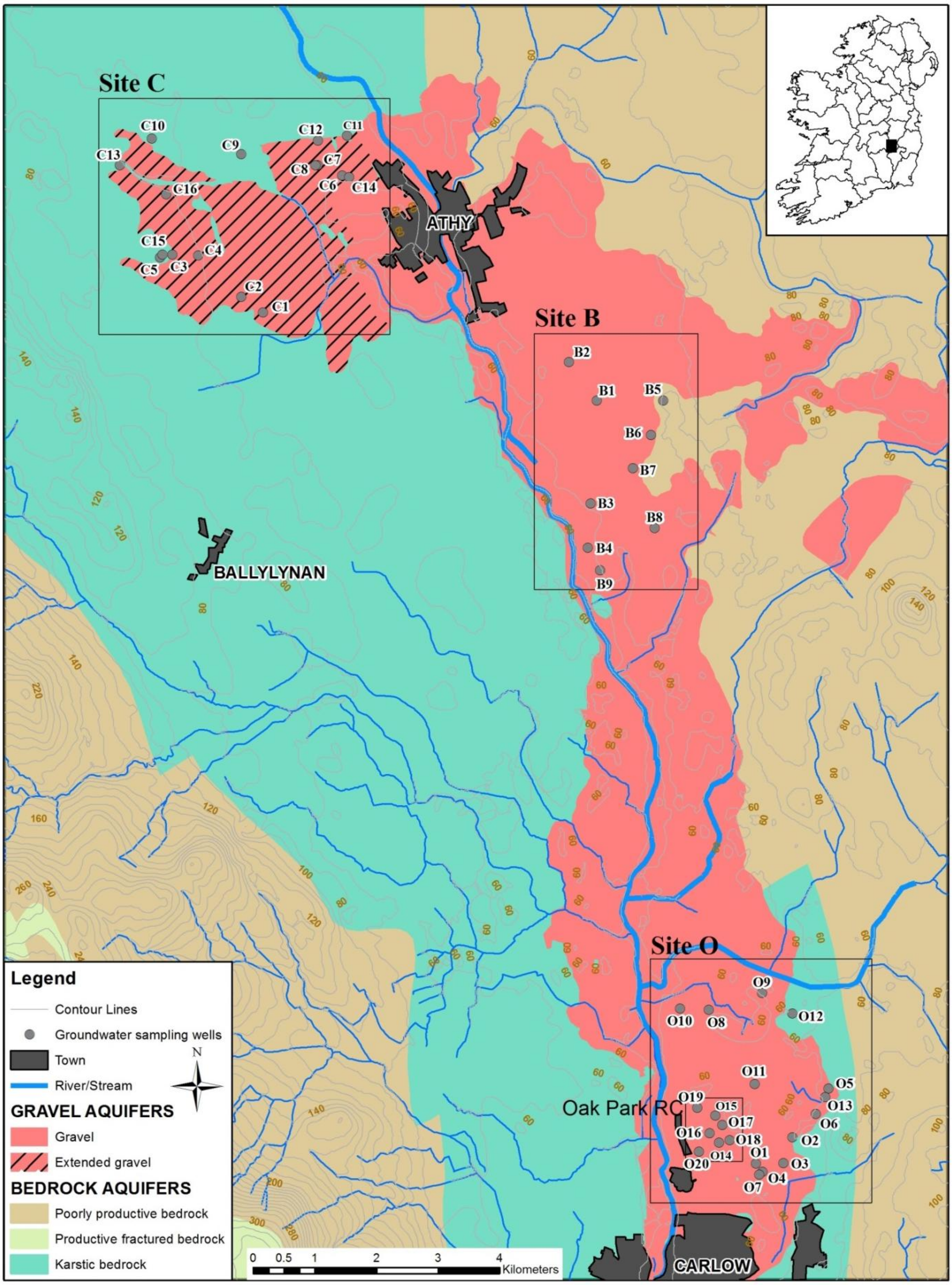

Figure 2

Figure 2

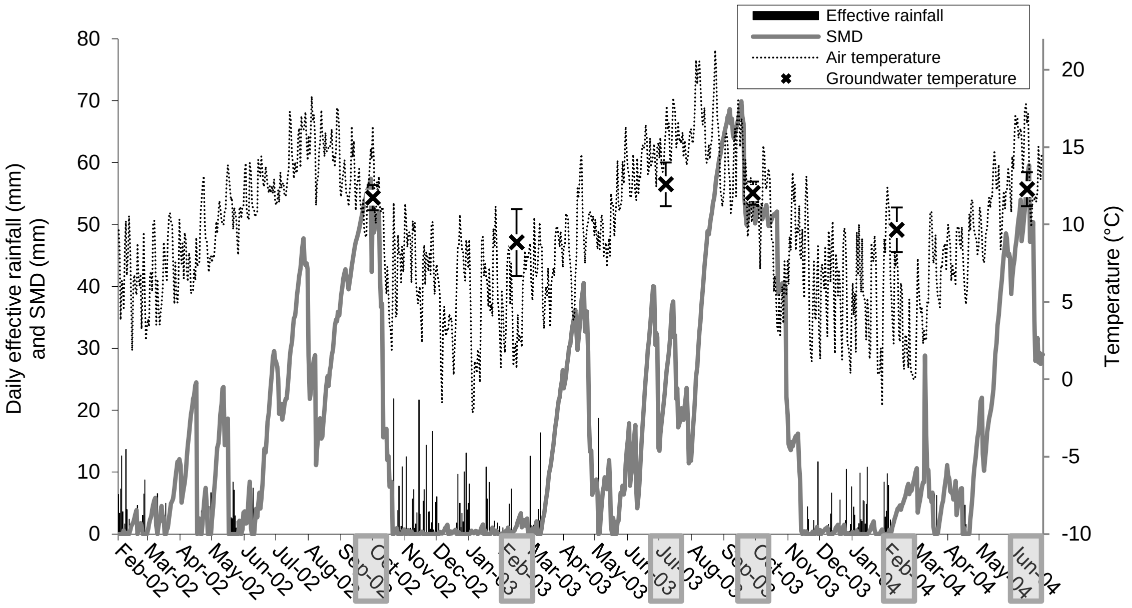

Figure 3

Figure 3

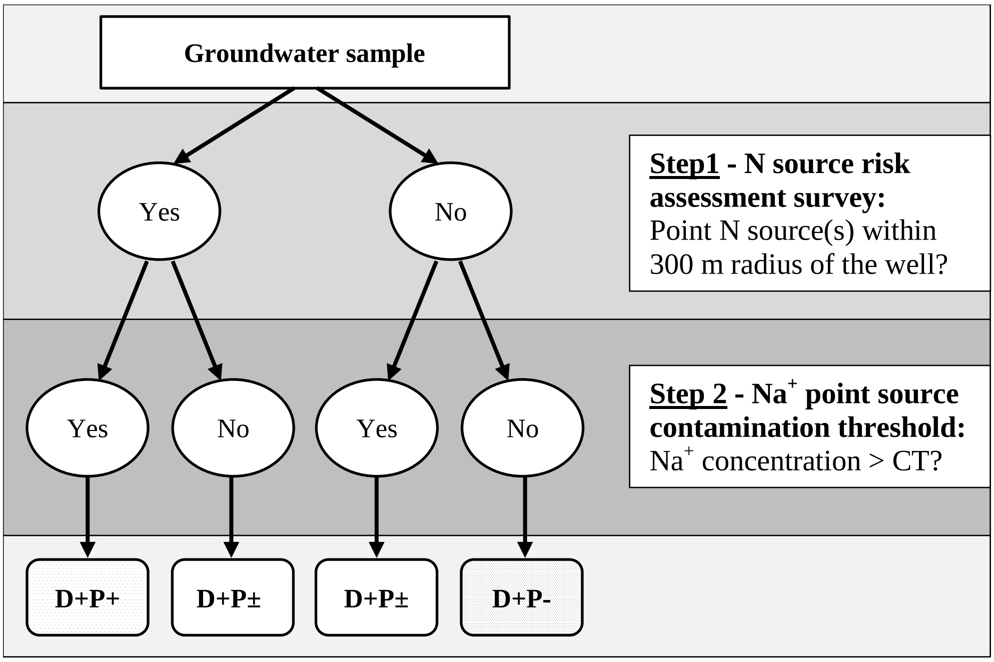

Figure 4

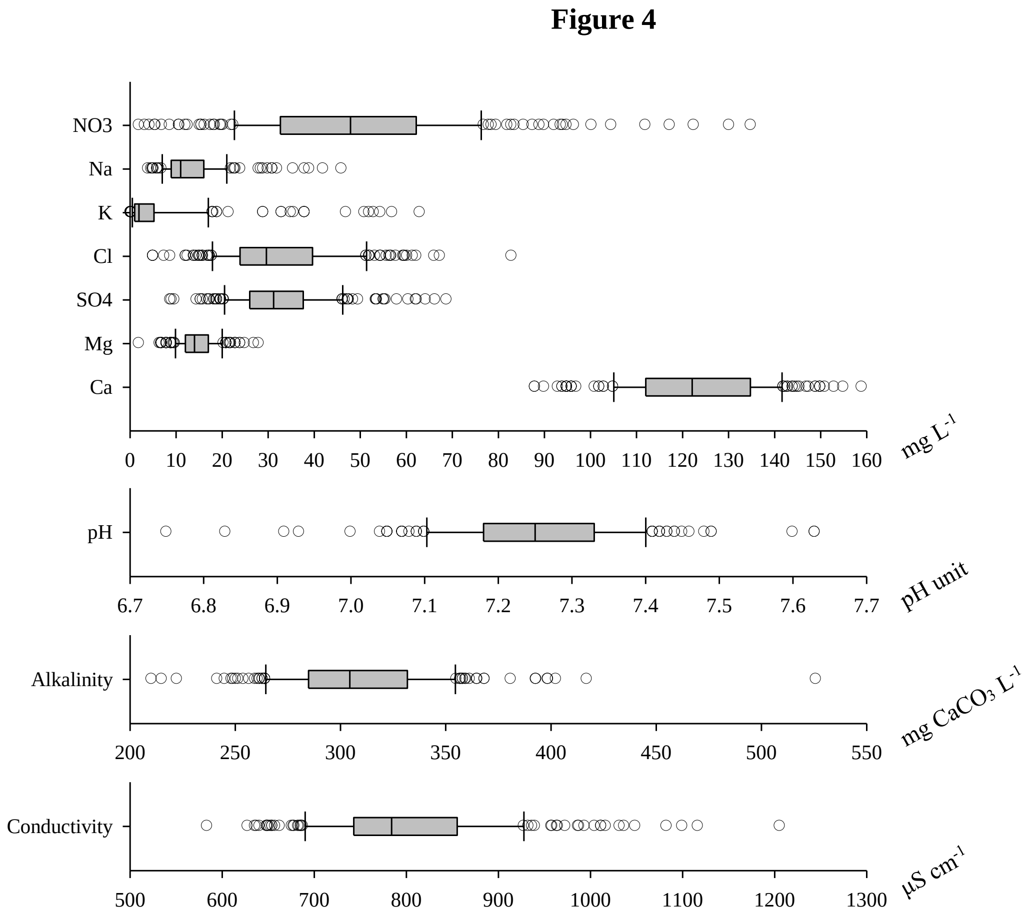
Figure 4
NO3
Na
K
Cl
SO4
Mg
Ca
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
mg L-1
pH
6.7
6.8
6.9
7.0
7.1
7.2
7.3
7.4
7.5
7.6
7.7
pH unit
Alkalinity
200
250
300
350
400
450
500
550
mg CaCO3 L-1
Conductivity
500
600
700
800
900
1000
1100
1200
1300
µS cm-1

Figure 5

Figure 5

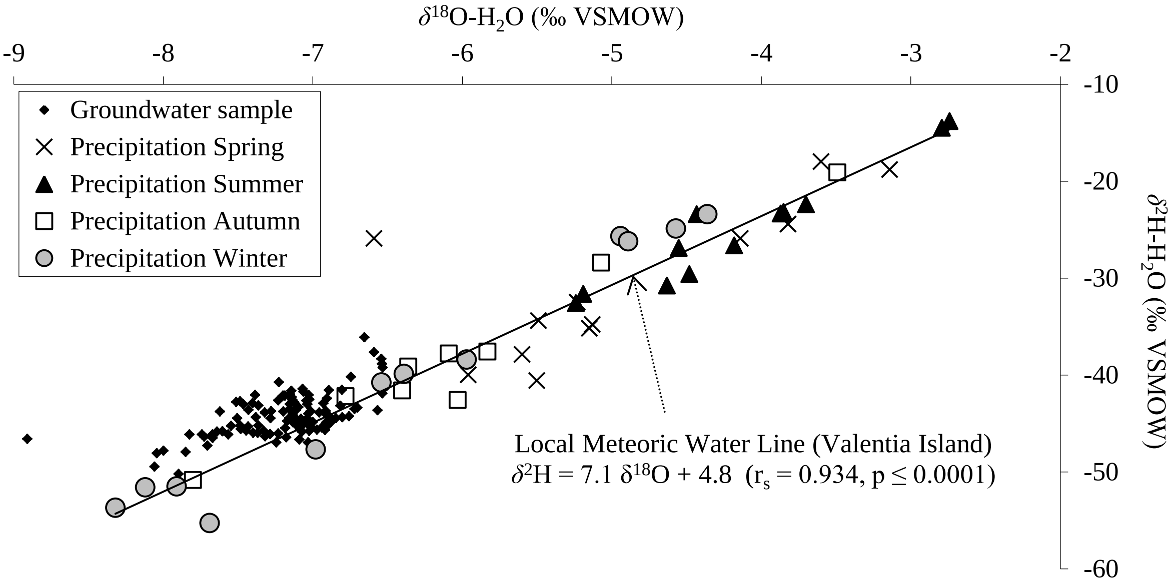

Figure 6

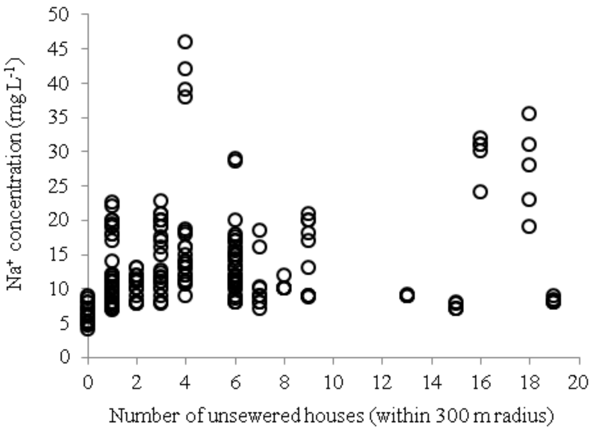
Na⁺ concentration (mg L⁻¹)
0
5
10
15
20
25
30
35
40
45
50
0
2
4
6
8
10
12
14
16
18
20
Number of unsewered houses (within 300 m radius)

Figure 7

Figure 7