A critical assessment of widely used techniques for nitrate source apportionment in arid and semi-arid regions.

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Abstract- The assessment of nitrate pollution origin using stable isotope techniques is a fundamental prerequisite for the application of sustainable groundwater management plans. Although nitrate pollution is a worldwide groundwater quality problem, existing knowledge on the origin of nitrate pollution in arid and semi-arid regions is still scarce. Using the example of the Grombalia aquifer (NE Tunisia), this work summarizes the main strengths and constraints of multi-isotope techniques targeting at nitrate source identification and apportionment The results highlighted that, even in the case of well-established methodologies, like those of isotope hydrogeochemistry ($\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$ and $\delta^{11}B$) and mixing modelling for source apportionment, it is fundamental to take into account regional and local end-members to avoid biased data interpretation and to fully exploit the potential of such accurate tools.
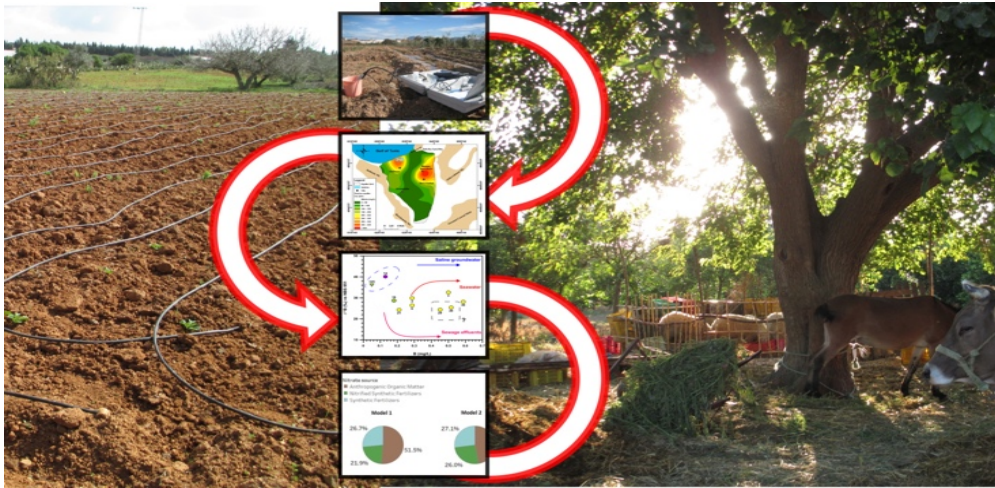
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27   **Graphical Abstract**.

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Introduction

Nitrate contamination of groundwater bodies is a widely acknowledged environmental and public health issue, although the correct procedure to be adopted for source identification is still an open question, especially when multiple sources (urban and rural) are present (Spalding and Exner, 1993). In arid and semiarid regions (A&SAR), groundwater is often characterized by high salinity (Greene et al., 2016; Jalali, 2007; Leduc et al., 2018) and diffuse nitrate contamination (Alsharhan and Rizk, 2020; Gutiérrez et al., 2018), with mutually interacting processes. Indeed, even if the anthropogenic recharge by sewage and irrigation water has a negligible effect in terms of salinity input, the associated nitrate content fuels up water-rock interaction processes, eventually leading to a further mineralization increase. This mechanism makes nitrate source apportionment even more difficult with hydrochemical and isotopic tools (Re and Sacchi, 2017). In addition, it is of paramount importance not only to correctly identify the pollution source(s), but also to assess the predominance of one source over another, if a simultaneous contribution of multiple sources occur. This can be estimated using isotope mass-balance mixing models, which are based on δ^{15}N-NO3 and δ^{18}O-NO$_3$ values (Deutsch et al., 2006; Phillips and Gregg, 2003; Phillips and Koch, 2002; Voss et al., 2006). The application of these models has proved to be complicated especially in the case of diffuse contamination, because of the temporal and spatial variability in δ^{15}N and δ^{18}O of NO$_3$, and the possible isotope fractionation processes (e.g. denitrification) that alter the isotopic composition of the initial source signal and the chemical concentrations of Nitrogen species (Moore and Semmens, 2008; Xue et al., 2009a). Additional parameters like δ^{11}B may help overcome the difficulty of overlapping nitrate isotope signatures. The rationale for a coupled use of B and N isotopes is that these elements have the same anthropogenic origin and co-migrate in the groundwater. Nevertheless, boron is always present at a background concentration that mostly depends on the aquifer matrix, influencing also its isotopic composition and potentially masking the signature of the

58 anthropogenic input (Martinelli et al., 2018; Re and Sacchi, 2017) . Bayesian-based stable isotope

59 mixing models, like SIAR or MixSIAR (Parnell et al., 2010; Stock et al., 2018), have been widely used

60 to determine the probability distribution of the proportional contribution of each nitrogen source

61 to a mixture and to overcome the constraints mentioned above (e.g. (Matiatos, 2016; Meghdadi

62 and Javar, 2018; Xia et al., 2017; Yang et al., 2013; Zhang et al., 2018)). Results of these models are

63 strongly dependent on the values assumed for $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ of the contamination sources.

64 Comparing field data with the compositional ranges reported in the literature (e.g. (Kendall et al.,

65 2008; Kendall and Aravena, 2000)) can provide useful insights on the possible sources of

66 contaminations. However, given the recent increase in studies addressing NO_3 contamination and

67 the growing accessibility of analytical techniques, the compositional ranges classically reported in

68 the $\delta^{18}O_{NO3}$ - $\delta^{15}N_{NO3}$ biplot are getting broader and often overlapping. This can make difficult the

69 interpretation unless one carries out a more in-depth analysis by comparing the results of similar

70 geographic and climatic areas. Finally, little is known about the real effectiveness of this approach

71 in A&SAR, due to the potential modifications of the isotopic input signal related to the long N

72 residence time in soils (Craine et al., 2015).

73 A&SAR cover almost the 41% of Earth's land surface and are home for approximately 35% of the

74 global population (Mortimore et al., 2009). In these areas, groundwater often represents the main

75 freshwater source to support local populations' development (Re and Zuppi, 2011), and climate

76 changes may further reduce its availability, as per the increase of the length of dry season and

77 extreme droughts events (IPCC, 2021). Hence, the identification of the proportional contribution of

78 nitrate sources is essential to control nitrate pollution and to develop effective management

79 practices.

80 This paper aims at evaluating the effectiveness of the more widely used approaches targeted to the

81 identification of nitrate contamination origin in A&SAR, when multiple inputs are present and the

82 long-term sustainability of groundwater resources is at stake. To this end, a case study,

83 representative of typical nitrate contamination issues Mediterranean coastal aquifer is presented:

84 the Grombalia aquifer (Tunisia).

85

86 **Material and methods**

87 **Study area**

88 The Grombalia coastal aquifer (Tunisia) covers a surface of 719 Km². It is constituted by a shallow

89 aquifer, with an average thickness of about 50 m, hosted in the Quaternary continental sand, clayey

90 sand and sandstones deposits, and multilayers confined aquifer with average thickness of about 100

91 m communicating through discontinuities (Castany, 1948). The recharge in the shallow unconfined

92 aquifer mainly occurs in the pediments of the surrounding mountains and converges to the central

93 part of the basin. There, a general southeast–northwest flow carries groundwater to the Gulf of

94 Tunis discharge areas (Ben Moussa et al., 2010; Gaaloul et al., 2014).

95 The Grombalia aquifer is located in one of the most important agricultural regions in the country,

96 and groundwater is often used for both irrigation and domestic consumption (Tringali et al., 2017).

97 However, the high salinity and nitrate concentrations make the shallow aquifer unsuitable for

98 human use without treatment (Ben Moussa and Zouari, 2011; Charfi et al., 2013; Kammoun et al.,

99 2018b), as many other aquifers in the country and in the Mediterranean basin (Leduc et al., 2018).

100 Recently, (Re et al., 2017) demonstrated that high nitrate concentrations are present also in the

101 deeper aquifer, generally considered less vulnerable to pollution, and that synthetic fertilizers are

102 not the only source of nitrate contamination, pointing out the dual impact of both agricultural and

103 domestic sources. However, so far no study permitted to distinguish between manure and sewage

104 input, nor to determine the proportional contribution of each nitrate source to groundwater of

105   these systems. The latter would be a crucial point to devise adequate new policies and management

106   strategies for preventing the aquifer from further contamination.

107

108   **Hydrochemical and isotopic analyses**

109   A total of 116 samples were collected in both the shallow (n=61) and deep (n=55) Grombalia

110   aquifers in different campaigns (February 2014, September 2014 and March 2015; Figure S1), to

111   assess the possible occurrence of seasonal variations and changes in groundwater quality. *In situ*

112   measurements of electrical conductivity, pH and water temperature were performed, using a WTW

113   340i multimeter. Samples for major ion analysis were filtered through 0.45 µm cellulose membrane

114   and stored in high density polyethylene bottles. Chemical and isotopic analyses of the water

115   samples were performed at the Laboratory of Radio-Analyses and Environment (LRAE) of the

116   National School of Engineers of Sfax (Tunisia). Major elements were analysed using a Dionex DX 100

117   ion chromatograph equipped with a CS12 and an AS14A-SC Ion Pac columns and an AS-40auto-

118   sampler. The total alkalinity (as HCO_3^-) was determined by titration with standard hydrochloric acid

119   (0.1 N) using phenolphthalein and methyl orange and as indicators. The error, based on the charge

120   balance, was calculated to be < 5%. Analyses of the stable isotopes of the water molecule (δ^2H and

121     $\delta^{18}O$) were performed using the Laser Absorption Spectrometer LGR DLT 100 (Penna et al., 2010).

122   Results are reported in ‰ vs. SMOW (Standard Mean Oceanic Water) with an analytical error ± 1

123   for δ^2H and ± 0.1 for $\delta^{18}O$. The isotopes of dissolved nitrate ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) were prepared and

124   analysed at the ISO4 private laboratory (Turin, Italy) using a Finningan™ MAT 250 Mass

125   Spectrometer, following the procedures described by Silva et al. (Silva et al., 2000). Results are

126   expressed in ‰ and refer to AIR and V-SMOW (Gonfiantini et al., 1995) with uncertainties (2σ) of

127   ±0.5‰ and ±1‰ respectively. Boron concentration was determined by ICP-AES. Boron isotopes

128 (expressed as $\delta^{11}B$‰vs NBS951) were determined by MC-ICP-MS at ALS Scandinavia AB, Sweden,

129 with combined uncertainty (1σ) of ±0.4 to ±1‰.

130

131 **Multivariate Statistical Analysis**

132 Statistical data treatment, in the form of Principal Component Analysis (PCA; e.g. (Chatfield, 2018))

133 and Hierarchical Cluster Analysis (HCA), was performed using the statistical package SPSS 15.0 for

134 Windows® (SPSS, Inc., Chicago, IL, USA, 2004). PCA was conducted on a total of 115 samples

135 considering only the hydrochemical and isotopic variables (n = 13) common to all samples (pH, HCO_3,

136 EC, TDS, Cl, NO_3, SO_4, Na, K, Mg, Ca, $\delta^{18}O$, δ^2H). To increase the efficiency of the PCA, pH and HCO_3

137 were excluded from the analysis due to a poor correlation with most of the variables. To reduce the

138 overlap of the original variables over each principal component a Varimax rotation was

139 performed(Kaiser, 1958).

140 HCA was conducted using Ward's single linkage method in combination to Euclidean distance, in

141 order to identify groundwater groups showing similar hydrogeochemical composition.

142

143 **Bayesian isotope mixing model**

144 To estimate the proportional contributions of multiple NO_3 sources, a Bayesian isotope mixing

145 model was applied using the software package SIAR (Stable Isotope Analysis in R), a language and

146 environment for statistical computing (Jackson et al., 2009; Moore and Semmens, 2008; Parnell et

147 al., 2010). For a set of N mixture measurements on J isotopes with K source contributors, the mixing

148 model is expressed as follows (Equation 1; (Parnell et al., 2010)):

149

150 *Equation 1*

$$X_{ij} = \sum_{k=1}^{K} p_K \left(S_{jk} + C_{jk} \right) + \varepsilon_{ij}$$

151

$$S_{jk} \sim N(\mu_{jk}, \omega_{jk}^2)$$

152

$$C_{jk} \sim N(\lambda_{jk}, \tau_{jk}^2)$$

$$\varepsilon_{jk} \sim N(0, \sigma_j^2)$$

where X_{ij} is the isotope value j of the mixture i, in which i=1, 2,3,.,N and j=1, 2, 3,., J; S_{jk} is the source value k on isotope j (k=1, 2, 3,., K) and is normally distributed with mean μ_{jk} and standard deviation ω_{jk}; p_k is the proportion of source k, which needs to be estimated by the SIAR model; c_{jk} is the isotope fractionation factor for isotope j on source k and is normally distributed with mean λ_{jk} and standard deviation τ_{jk}; and ε_{ij} is the residual error representing the additional unquantified variation between individual mixtures and is normally distributed with mean 0 and standard deviation σ_j. More information about the model can be found in (Jackson et al., 2009; Moore and Semmens, 2008).

Results and Discussion

This section is organized so as to follow the most commonly adopted investigation approaches, in increasing degree of complexity and analytical cost involved. For each step, the main findings, uncertainties and criticalities are evidenced, stressing the difficulties encountered when applied to A&SAR.

**The basics: nitrate contents and distribution**

The hydrogeochemical assessment confirms that high nitrate concentrations characterize both the shallow and the deep aquifers regardless to the season and/or irrigation periods (Table S1). Overall, approximately 51% of the samples (62% in the shallow aquifer and 38% in the deep one) are unsuitable for drinking water purposes, having concentrations exceeding the statutory limits set by the WHO (50 mg[NO_3^-]/L (WHO, 2011)).

These findings are in line with the observed nitrate concentration increase, commonly observed in the past decades in aquifers from A&SAR, due to anthropogenic inputs (Gutiérrez et al., 2018).

178   Results also highlight the presence of two pollution hotspots, common to both the shallow and the

179   deep aquifers, as suggested by (Re et al., 2017) (Figure 1). The first one is located in the central part

180   of the plain, in an area identified by (Chenini et al., 2015) as at high to very high groundwater

181   contamination risk, due to the dominance of irrigated areas. The second hotspot is located along

182   the coast, where urban settlements and industrial activities prevail. While this seems to suggest the

183   dominance of only two contamination sources (agriculture and urban/domestic activities), the lack

184   of sanitation facilities in some rural and peri-urban zones represents another potential

185   anthropogenic contribution to nitrate pollution in the region (Re et al., 2017). The latter can be

186   hardly discriminated by solely integrating hydrochemical and land use information. The similarity in

187   the distribution of pollution hotspots in both aquifers suggests their interconnection and highlights

188   their vulnerability.
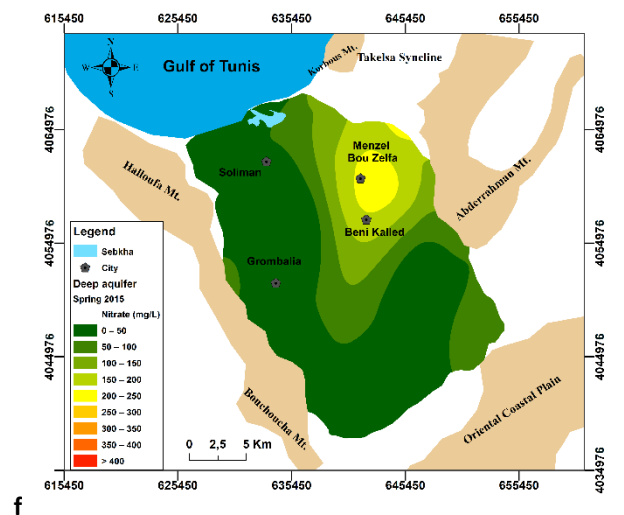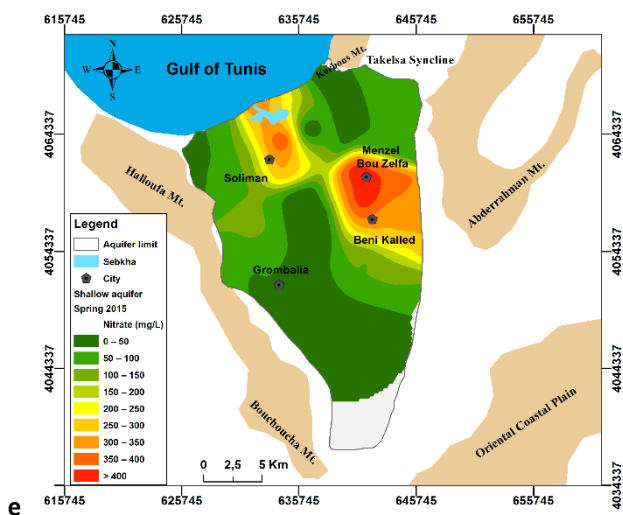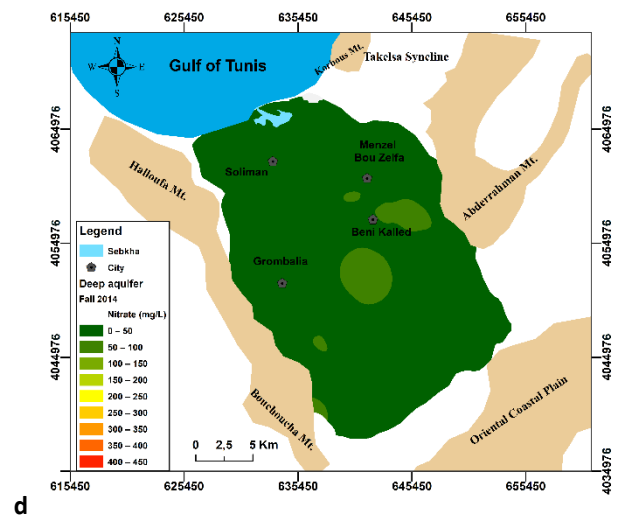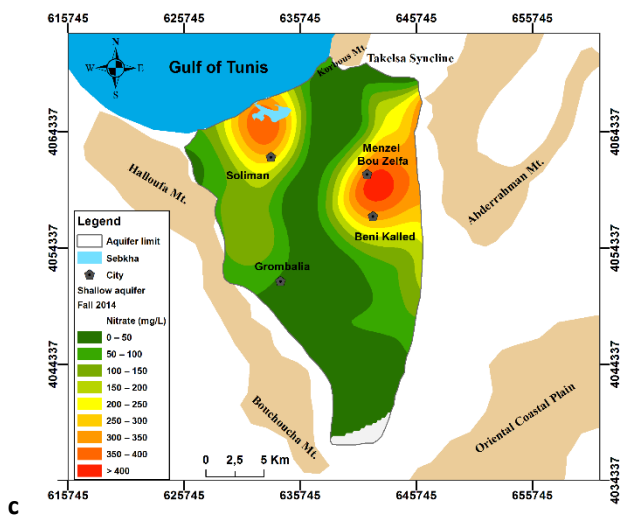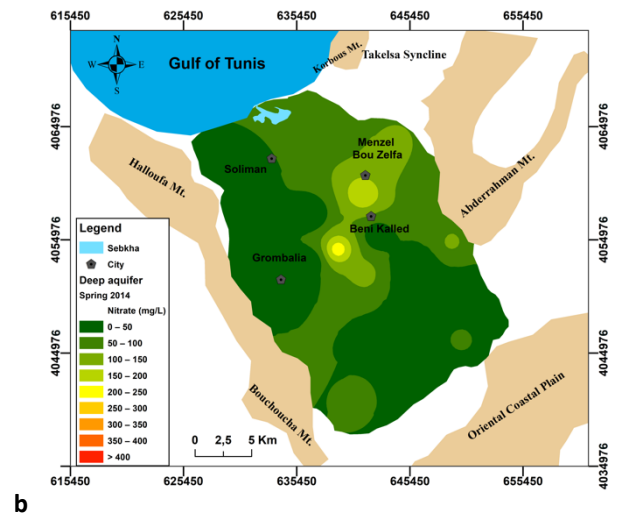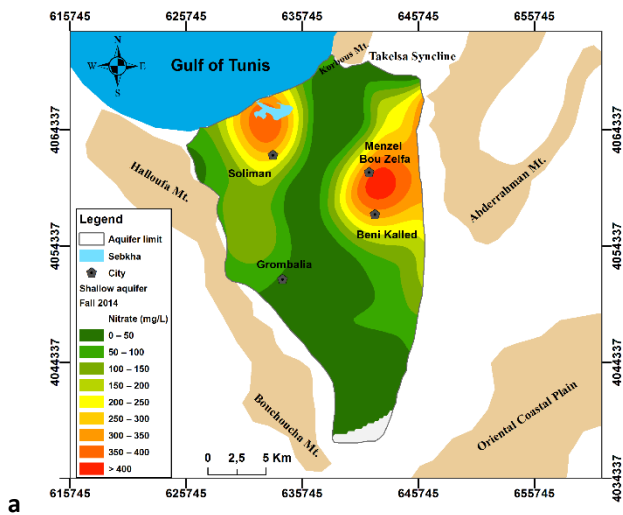
Figure 1. Nitrate concentrations (in mg/L) distribution maps in the different sampling seasons: a) shallow aquifer, spring 2014; b) deep aquifer, spring 2014; c) shallow aquifer, fall 2014; d) deep aquifer, fall 2014; e) shallow aquifer, spring 2015; f) deep aquifer, spring 2015.

Multiparametric approach: advanced statistical analysis

PCA was performed to support geochemical evidences and to identify common patterns among samples. The value of 2183.76 for the Barlett's chi-square sphericity test (55 degrees of freedom and a minimum significance level of 0.00) indicates the existence of a statistically significant interrelationship between variables. Moreover, the measure of sampling adequacy obtained by the Kaiser Meyer Olkin statistic returns a rather high value (0.710), validating the PCA application. From the analysis, two varifactors (VF) were extracted (Table S2, Figure 2a), explaining 78.6% of the total variance, and supporting the hypothesis of the co-existence of two main processes dominating groundwater hydrogeochemistry: salinization and NO_3 pollution.

In particular, VF1, accounting for 60.4% of the total variance, indicates a strong positive correlation of EC, TDS, Cl, SO_4, Na, K, Mg, Ca, and is interpreted as the salinization component, attributable to water-rock interaction processes and seawater intrusion. This result is in agreement with literature data (e.g. (Gaaloul et al., 2014; Re et al., 2017; Slama and Sebei, 2020)) highlighting that natural salinity can be associated to the dissolution of evaporites throughout the plain, and to saline intrusion in the shallow wells near to the coast.

On the other hand, the second component (VF2), explaining 18.2% of the total variance, shows a strong correlation with δ^2H, $\delta^{18}O$, and NO_3. This factor is representative of nitrate pollution, either associated to direct NO_3 input (e.g. fertilizers, manure and sewages), or to the remobilization of NO_3 stocked in the unsaturated zone associated to irrigation activities (i.e. return flow of evaporated irrigation waters, explaining the correlation between isotopic enrichment and NO_3 concentrations in VF2).
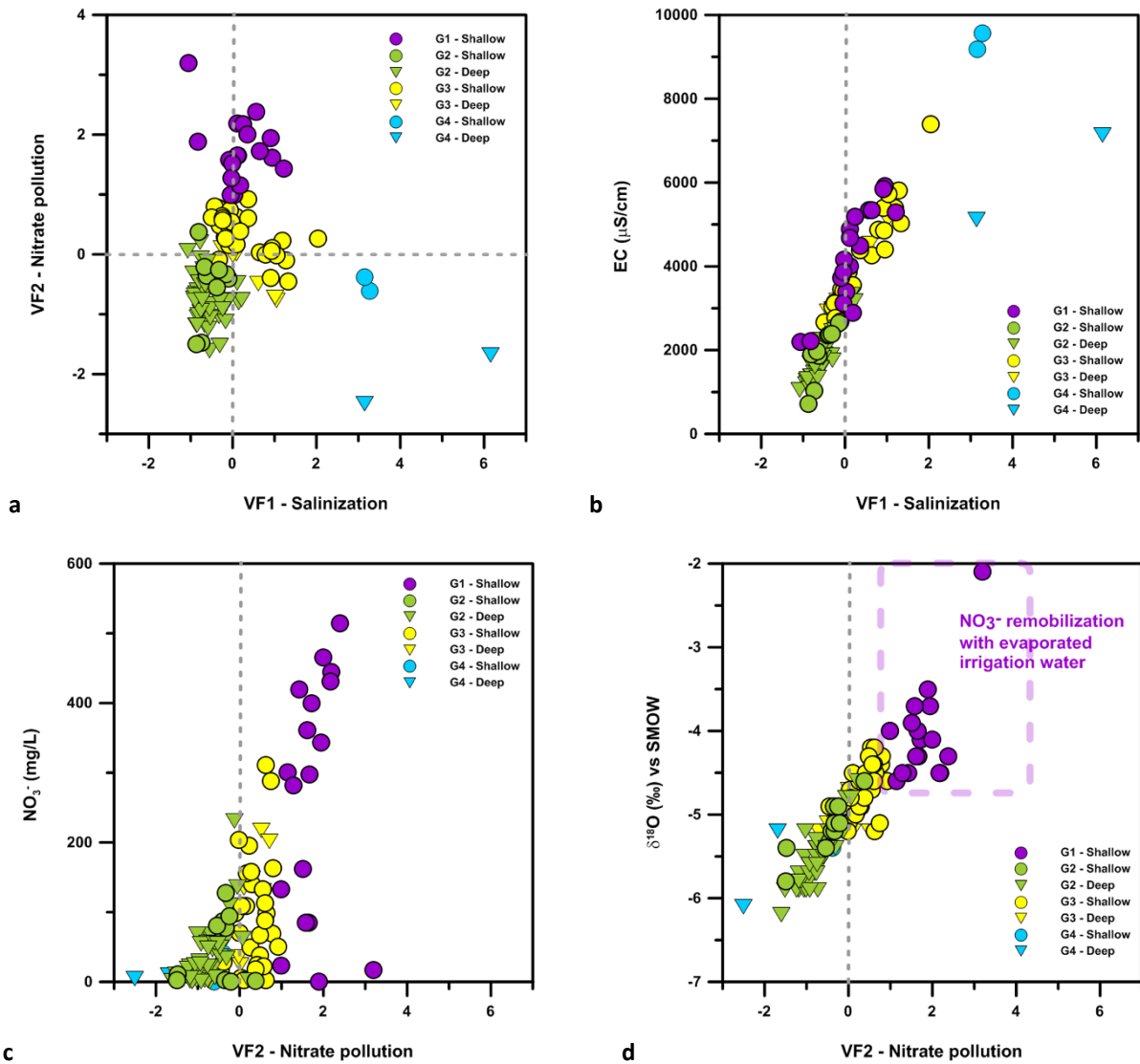
Figure 2. Relationship between the varifactors related to HCA groups (Ward method) and the main variable they represent. a) VF2 (NO₃ pollution) versus VF1 (Salnization); b) Electrical Conductivity (EC) versus VF 1; c) NO₃⁻ concentration versus VF2; d) δ¹⁸O versus VF2.

The HCA permitted to classify the samples into four groups (Table S1), and this classification was used to further constraint the dominant process in each sample (Figure 2):

- The first group (G1; 15.5% of the samples) includes the samples where NO_3 remobilization associated to irrigation practices dominates, as evidenced by the highest scores for $\delta^{18}O_{H2O}$. Interestingly, this cluster includes only shallow well samples (29.5% over the total samples collected in the shallow aquifer), highlighting the previously mentioned high vulnerability to pollution of the shallow aquifer.

12

- On the other hand, most of the samples collected in the deep aquifer (77.8%) fits in a second group (G2; 45% of the total samples) characterized by more negative $\delta^{18}O$ and δ^2H values, negative values of both VF1 and VF2, and lower average NO_3 concentrations (38.4 mg/L). Additionally, the 33.3% of the deep aquifer samples belonging to this group show NO_3 concentrations exceeding the statutory limit for drinking waters (maximum concentration = 230.6 mg/L). This result confirms the vulnerability of the deeper aquifer, also evidenced by the general chemistry, and points out that nitrate pollution origin in the deep aquifer should be attributed to a different process than NO_3 remobilization.

- The third group (G3; 35.6% of the samples) does not show a clear dominance of salinization nor nitrate pollution. In particular, in this group, 50.1% of the shallow well samples is found, highlighting common issues affecting aquifers located in A&SAR, where the high natural salinity, due to evaporation, water rock-interaction processes and/or seawater intrusion(Re et al., 2017), (Kammoun et al., 2018a), makes difficult the correct discrimination of salinization sources. Hence, this group could represent samples affected by mixing processes, without a remarkable dominance.

- As concerns salinization, this process appears to be dominant over NO_3 pollution only in 4 points out of 115 (G4).

In summary, while the multiparametric statistical approach permits to further highlight the previously mentioned direct and indirect contribution to nitrate pollution, it is less performant to correctly discriminate among the dominant processes affecting samples' composition (i.e. natural versus anthropogenic salinization), and hence may not be sufficient to provide information for the correct identification of nitrate pollution sources.

In-depth analysis: multi-isotopic assessment

In investigations targeted to constraint nitrate pollution origin, the dual nitrate isotopes ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) approach is generally recognized to be a quite powerful tool ((Re and Sacchi, 2017) and references therein). By comparing $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ of the groundwater samples collected in the Grombalia aquifer with the composition of the main sources reported in the literature (Kendall et al., 2008) (Figure 3), it is possible to observe that, most of the samples plot in the compositional range of soil organic matter (regardless of the membership of one of the groups previously identified with the HCA). However, as pointed out by (Re et al., 2017), due to the NO_3 concentrations exceeding the expected natural background level (10-12 mg/L (Edmunds and Shand, 2009; Sacchi et al., 2013)), these samples likely record a mixed contamination from both synthetic fertilizers and anthropogenic organic matter (animal or human waste). The latter source is characterized by enriched $\delta^{15}N_{NO3}$ values, exceeding +10‰; nevertheless, this organic matter contribution may be considered dominant for samples showing a $\delta^{15}N_{NO3}$ greater than+8.6‰ (Re and Sacchi, 2017). These findings, common to many other aquifers in A&SAR (Alsharhan and Rizk, 2020; Danni et al., 2019; Diédhiou et al., 2012; Favreau et al., 2009; Gutiérrez et al., 2018), highlight the need for a more specific assessment of NO_3 isotopic ranges. In particular, the use of locally relevant $\delta^{15}N$ source values is generally recommended (Isonitrate, 2010), but probably still not sufficient to unveil the specific impact of environmental and climatic conditions to soil organic matter, fertilizers, manure and sewage during their residence time in the unsaturated zone, prior to groundwater recharge. (Kendall, 1998) introduced the expected ranges of $\delta^{15}N$ and $\delta^{18}O$ values of nitrate sources through visual $\delta^{18}O$ vs $\delta^{15}N$ cross plots and these have been updated several times (Kendall et al., 2015, 2008; Xue et al., 2009b). However, the identification of the origin of nitrogen concentrations in groundwater only by using nitrate isotope techniques is complicated due to the presence of mixed input from point and non-point sources and the occurrence of biogeochemical processes (e.g.,

nitrification, denitrification), in the unsaturated zone and the water body, that may alter the initial

isotopic values of the nitrate sources (Chen et al., 2010; Curt et al., 2004; Kendall, 1998). Denitrification is a process that results in an increase of nitrate $\delta^{15}N$ and $\delta^{18}O$ as concentration decreases (Mariotti et al., 1981). The observed linear relationship between the $\delta^{15}N$ and $\delta^{18}O$ values of the sampling points, as depicted in Figure 3, implies that denitrification probably occurred for the wells: 6 and 26 sampled in spring 2014; 2 and 114 sampled in fall 2014; and 6 sampled in spring 2015 (Table S1).

Pending further developments in the delineation of the sources, a useful approach to support the interpretation of isotopic hydrochemistry can come from socio-hydrogeology (Re, 2015). The administration of structured interviews to wells' owners during *in situ* measurements, can provide useful insights on the manure and fertilizers use (Tringali et al., 2017), and successfully complement isotopic data (Re et al., 2017).
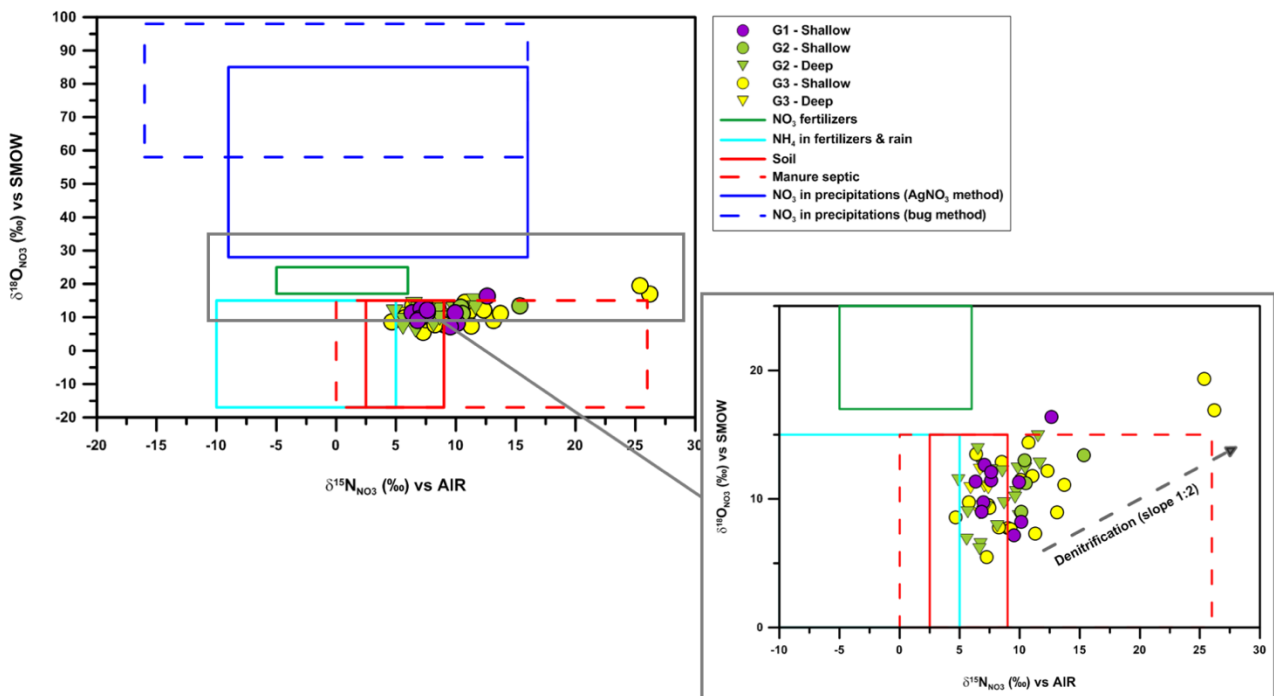
Figure 3. Stable isotope composition of dissolved nitrates in groundwater from the Grombalia Basin, with ranges for groundwater as per (Kendall et al., 2008).

290 However, an open question still remains related to the distinction between manure and sewage

291 derived nitrates, due to their overlapping $\delta^{15}N_{NO3}$ values (~10–15‰ (Kendall et al., 2008)). To this

292 end, selected samples with enriched $\delta^{15}N_{NO3}$ and mostly belonging to the G3 group, were also

293 analysed for $\delta^{11}B$ (Table 1).

294 As in the case of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$, the comparison of $\delta^{11}B$ of samples with references data, and

295 more specifically with the compositional fields reported in the literature (Tirez et al., 2010; Widory

296 et al., 2005, 2004) (Figure 4), often provides important insights on contamination origin. In the case

297 of the samples collected in the Grombalia aquifer however, some data could bias the appropriate

298 source identification, if not correctly interpreted and/or supported by an adequate land use

299 analysis,. For example, one sample (26; $\delta^{11}B$ = 25.45 ‰) falls in the compositional range of hog

300 manure, even if the well is located in a region where pig farming is not present (as this is not a

301 dominant activity in the country).

302

303 *Table 1. Summary of the main features of the wells whose samples collected in spring 2015 were analyzed for $\delta^{11}B$. Ranges are*

304 *calculated for Electrical Conductivity values (as a proxy for salinity) and nitrate concentrations, based on the following percentiles:*

305 *(+++): ≥ 75; (++): ≥5O and <75; (+) ≥ 25 and <50; (-) < 25. Variability refers to the occurrence of significant variations in NO_3*

306 *concentrations in the different sampling campaigns.*

Well number	Area	Aquifer	Approx. distance from the sea (Km)	Salinity	Nitrates	Variability	$\delta^{11}B$ (‰)
2	Peri-urban	Shallow	3.0	++	++	NO	29.92
3	Peri-urban	Shallow	3.5	+++	++	YES	32.62
6	Rural	Shallow	9.3	+++	++	NO	26.41
14	Peri-urban	Shallow	11.8	+++	+++	NO	40.11
19	Rural	Shallow	13.7	+	++	NO	28.69
21	Rural	Shallow	17.9	++	++	YES	24.48
26	Rural	Shallow	18.5	++	+	NO	25.45
29	Rural	Shallow	12.7	++	++	NO	24.42
30	Peri-urban	Shallow	2.7	+++	+	NO	28.19
125	Rural	Deep	27.8	-	++	NO	36.10

307

308 Three samples (wells 3, 14, 125) plotting out of the literature manure compositional range, and

309 attributed to natural groundwater, show quite different hydrochemical characteristics, potentially

310 reflecting the interaction/mixing with different salinity sources (e.g. seawater and evaporites; Figure

311 4), and six samples (2, 6, 19, 21, 29, and 30) show an isotopic signature coherent with cattle manure.

312 However, as in the previous case, multiple sources are present (i.e. sewage, domestic effluents and

313 manure) that are not evidenced even by this coupled isotopic approach. Therefore, even though the

314 samples' selection for $\delta^{11}B$ analyses was set with the overall goal of disaggregating the

315 contamination end-members in the mixing group (G3), the results did not permit to achieve it.

316 Additionally, this comparison reinforces what previously emerged from the $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$

317 binary plot interpretation, and relates to the careful use of compositional ranges reported in the

318 literature. The latter indeed represent a valuable term of reference, but should not be considered

319 as the one and only landing point for data interpretation.

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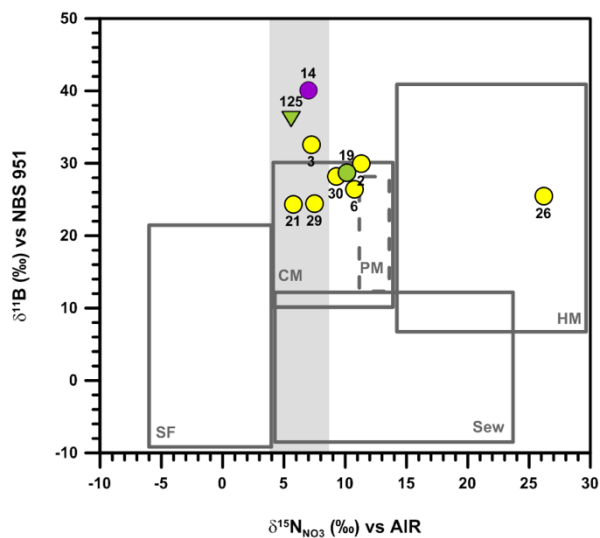
321

322 *Figure 4. $\delta^{11}B$ vs $\delta^{15}N$ of groundwater, for the samples collected in Fall 2015. Grey area = natural groundwater, SF: synthetic*

323 *fertilizers, Sew: sewage and septic plumes, CM: cattle manure, PM: poultry manure, HM: hog manure. (plot modified* (Sacchi et al.,

324 2013)*) compositional fields after* (Widory et al., 2005, 2004)*, and* (Tirez et al., 2010)*.*

325

326   To assist in the interpretation of $\delta^{11}B$ values, plots versus the B or the Cl content have been used in

327   the literature (Vengosh, 2014; Vengosh et al., 1994). In the case of our samples, the comparison

328   between $\delta^{11}B$ values and B concentrations permits to hypothesize the occurrence of seawater

329   intrusion in the wells located near to the coast (2, 3 and 30; Figure 5a) and to highlight the impact

330   of natural salinization in the recharge zone (well 125, and possibly to a lesser extent well 15, where

331   the high salinity is also due to high nitrate concentrations). However, it is interesting to notice that,

332   when comparing $\delta^{11}B$ values with Cl concentrations (Figure 5b) different hypothesis can emerge on

333   dominant processes. For example, the salinity of well number 3, where both the location near to

334   the coast and the interview administration outcomes (Tringali et al., 2017) supports the

335   aforementioned occurrence of saline water intrusion, could be attributed to the dissolution of

336   marine evaporites. Similarly, wells 14 and 125, both quite far from the coast (Table 1), are probably

337   affected by mixing with external saline sources (e.g. mixing with deeper brines/saline waters or

338   evaporates), rather than mixing with (modern) seawater. For this reason, in A&SAR where

339   evaporation processes and multiple sources of pollution can contribute to aquifer salinization, a

340   careful approach to the use of $\delta^{11}B$ literature data should be adopted when comparing results to

341   reference values in order to avoid a biased attribution of dominant sources and processes, especially

342   when B and Cl do not have a common origin.

343
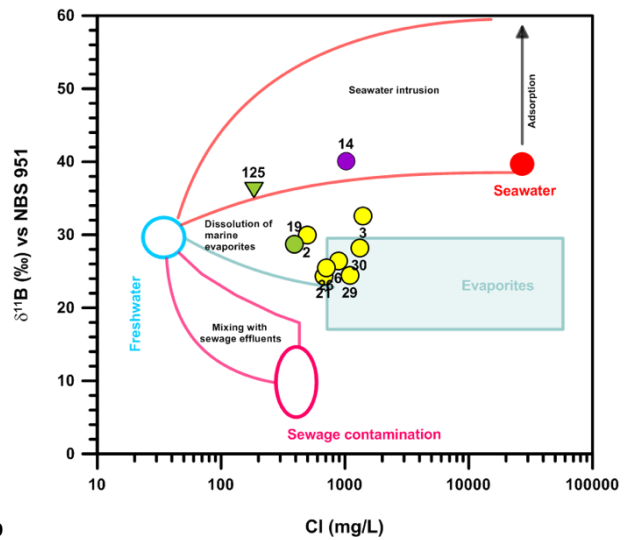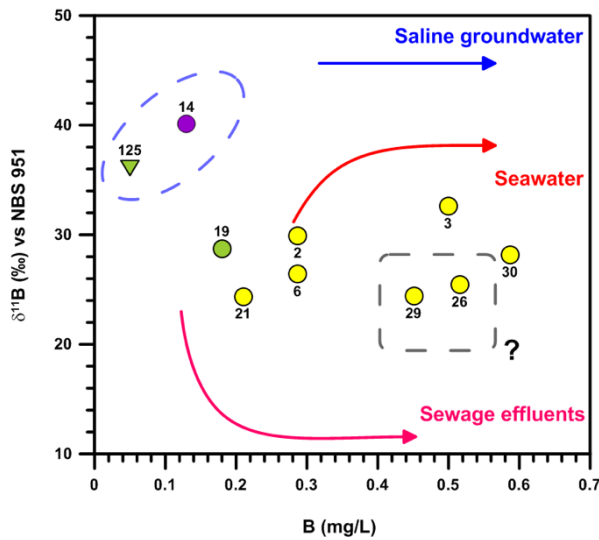
Figure 5. (a) δ11B versus B concentrations compared to the processes evidenced by (Vengosh et al., 1994); (b) δ11B versus Cl concentrations, modified after (Vengosh, 2014).

Results therefore show, as emerged in a similar geographical context (Re and Sacchi, 2017), that the high geochemical background for B (min [B]= 0.053 mg/L, max = 1.041 mg/L; average = 0.319 mg/l, Std Dev = 0.216) and Cl (min [Cl]= 57.6 mg/L, max = 2889.3 mg/L; average = 687.61 mg/l, Std Dev = 550.2), associated to complex water-rock interaction processes, limit the application of the coupled $\delta^{11}B$ and $\delta^{15}N$ isotopic systematics for the detection of groundwater nitrate pollution sources. In fact, despite the exceedingly high nitrate contents of both agricultural and civil origin, the depleted $\delta^{11}B$ values that characterize synthetic fertilizers (-10 to +20 δ‰) and sewage leakages (-9 to +12 δ‰) could not be detected. In this context, it may become fundamental not only looking at the potential sources of contamination, but also at the ongoing processes. In fact, if, on one hand, NO_3 input and nitrification can fuel up water-rock interaction processes and consequently favour carbonate dissolution due to acidification of the solution, on the other hand, denitrification produces alkalinity and fosters the re-precipitation of carbonates. While B incorporation into calcite enriches the solid phase in ^{11}B with respect to seawater, the dissolution of marine carbonates or of

360   secondary carbonates precipitated in the aquifers by freshwater would result in groundwater with

361   lower $\delta^{11}B$ values (Vengosh, 2010).

362   Additionally, cation exchange and carbonate dissolution trigger B sorption on clays, thus

363   contributing to the variation of the $\delta^{11}B$ composition of groundwater. Indeed, exchange or sorption

364   on clays favours ^{10}B and is accompanied by an increase in ^{11}B in the residual solution, potentially

365   leading to considerable ^{11}B enrichment coupled to a decrease in B content and in B/Cl ratios

366   (Kloppmann et al., 2015). This could be the case, for example of samples 14 and 125 collected in the

367   Grombalia aquifer. Another possible explanation, that may require further investigation, is the

368   presence of deeper and older waters (with high $\delta^{11}B$ and low B/Cl), originated from coastal air

369   masses and undergone limited water-rock interaction with the matrix, that is mixing with the more

370   recent waters located near to the recharge zone (e.g. well 125, approx. 28 Km from the coast).

371   In summary, several processes affect the Boron content and isotope systematics, contributing to

372   uncouple it from the nitrate isotope systematics and reducing its effectiveness for the identification

373   of the sources of nitrate contamination.

374

375   **Assessment of proportional source contribution using Bayesian isotope mixing modelling**

376   Two isotopes (j = 2) ($\delta^{15}N$ and $\delta^{18}O$ of NO_3) and three potential NO_3 sources (nitrified synthetic

377   fertilizers, synthetic fertilizers, and anthropogenic organic matter from manure and septic wastes)

378   were considered in this study to estimate the contribution of NO_3 sources in the sampling points of

379   the two aquifers. The three potential NO_3 source values were obtained from literature (Table 2).

380

381

382

383

384 *Table 2. End-members of $\delta^{15}N$ and $\delta^{18}O$ designated for the mixing models*

| Source	$\delta^{15}N$-NO_3 (‰)	$\delta^{18}O$-NO_3 (‰)	References
Nitrified synthetic fertilizers	+0.4±2.0 (model 1)	+4.0±2.0	(Kendall et al., 2008)
	+4.5 ±1.0 (model 2)	+4.0±2.0	This study
Synthetic fertilizers	+0.4±2.0	+22.0±2.0	(Kendall et al., 2008)
Anthropogenic organic matter OM (manure-septic wastes)	+12.0±3.0	+7.0±2.0	(Kendall et al., 2008)

385

386 As previously mentioned, since denitrification is limited to a few samples only, corresponding

387 experiments for determining enrichment factors of denitrification were not conducted in this study.

388 Thus, a fractionation factor of 26 ‰ for $\delta^{15}N$ and 12 ‰ for $\delta^{18}O$ were assumed in Equation 1, based

389 on (Divers et al., 2014).

390 Additionally, two models were run with a different value attributed to $\delta^{15}N$ of Nitrified Synthetic

391 Fertilizers (Table 2). Model 1 was run using an average value of +0.4±2.0 ‰ as per (Kendall et al.,

392 2008). In model 2 the end-member was chosen to be representative of the local conditions, thus a

393 value of +4.5±1.0 ‰ was selected based on the results of the current isotopic assessment (Figure 3,

394 Table S1). Indeed, in groundwater samples no $\delta^{15}N$ values lower than +4 were found. Synthetic

395 fertilizers and anthropogenic organic matter were left unchanged in both models, as there are not

396 evidences for a change in A&SAR.

397 The Bayesian isotope mixing model suggested the predominant sources of nitrate in the two

398 aquifers (Figure 6). There was a relatively higher contribution from anthropogenic organic matter in

399 the shallow aquifer in 2014 and 2015, compared to other two sources. The proportional

400 contribution of the aforementioned source in both models 1 and 2 increased in spring 2015

401 compared to spring of 2014 (~2-6 %), which implies a continuous increase in the application of

402 manure or release of septic wastes in the region. Nitrified synthetic fertilizers and synthetic

403 fertilizers are less profound sources of nitrogen pollution in the shallow aquifer, and their

404 proportional contribution is not constant throughout the year, because it is related to the adopted

405 fertilization strategies. The models also showed that anthropogenic organic matter is the major

406 source of nitrogen pollution for the deeper aquifer. The proportional contribution of organic matter

407 in the deep aquifer showed seasonality, with the highest values observed in spring and the lowest

408 ones in fall, which indicates that the impact of manure application or septic waste disposal is mostly

409 observed when the deep aquifer is recharged. As for the shallow aquifer, the contribution of nitrified

410 synthetic fertilizers and synthetic fertilizers is not as significant as the OM, and their proportional

411 contribution depends on the fertilization strategies followed. The SIAR model gives an estimation of

412 the proportional contribution, as it incorporates high uncertainty. The comparison of the two

413 models highlights the need to determine the local nitrate isotope end-members given that

414 estimates of the relative contributions of nitrate sources could be affected by any departures in

415 isotopic composition of potential sources and the values we used (Figure 6). For example, the two

416 models showed evident difference in the proportional contribution of the nitrate sources in the

417 deep aquifer system. However, both models ranked anthropogenic organic matter as the

418 predominant source of nitrogen pollution regardless the season and the year (Figure 6b). These

419 differences, relevant both at whole aquifer and single well (e.g. anthropogenic organic matter

420 ranked > 65% in samples 7 and 25 of the shallow aquifer, whereas nitrified synthetic fertilizers

421 contributed > 80% in samples 6 and 114 of the shallow and the deep aquifer, respectively) level,

422 may have important consequences if the mixing outcomes are used to support management

423 strategies for nitrate contamination reduction. The effect of the uncorrected attribution of the

424 dominant pollution sources may not only hamper the effective decrease in NO_3 levels in

425 groundwater, but also create a sense of discontentment of local stakeholders and decrease in

426 scientists and local institutions that are imposing constraints to local populations without meeting

427 the promised goals.
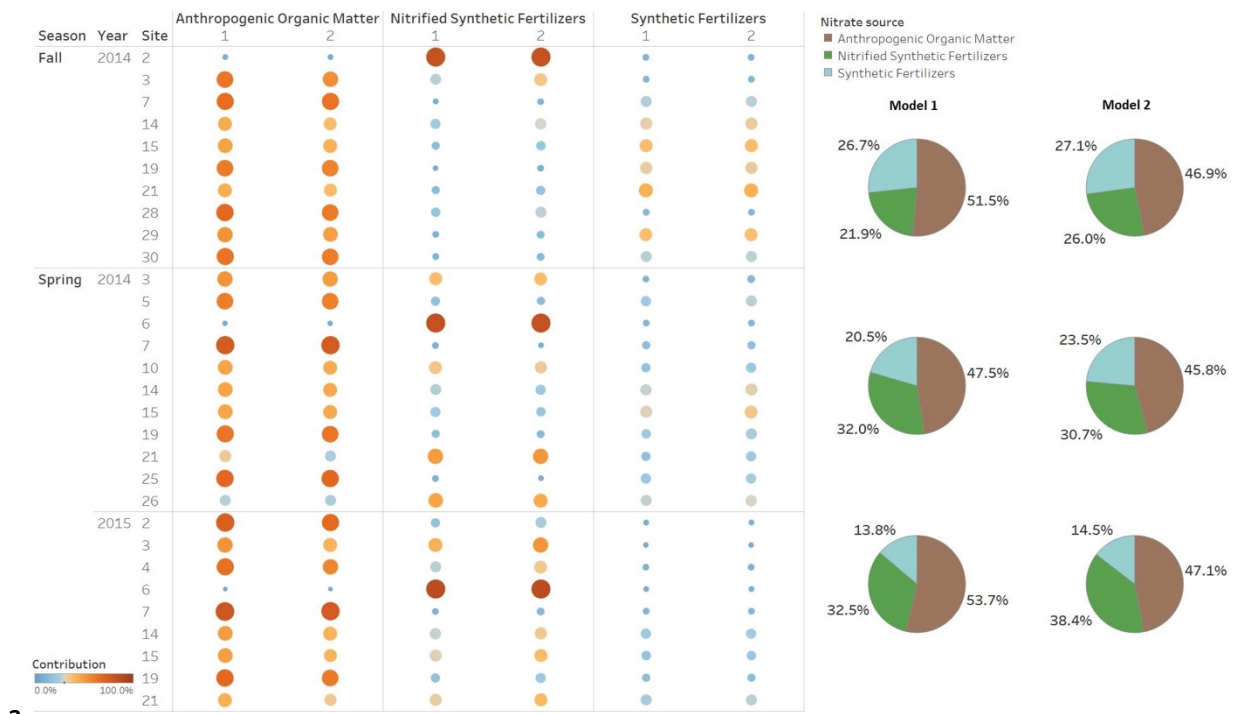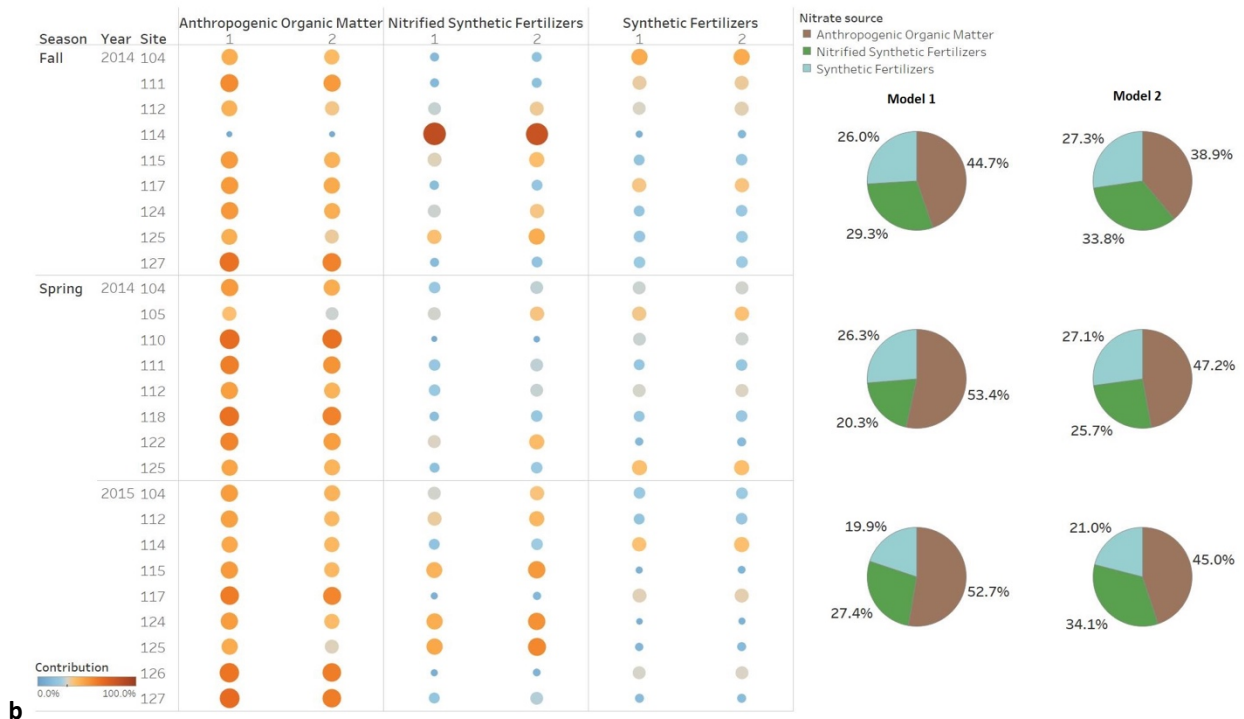
Figure 6. Comparison of the proportional estimated contributions of N sources (synthetic fertilizers, nitrified synthetic fertilizers, and anthropogenic organic matter) to nitrate in the shallow (a) and deep (b) aquifer sampled in 2014 and 2015. Model 1: using literature data $\delta^{15}N$ values from synthetic fertilizers; Model 2: $\delta^{15}N$ value for synthetic fertilizers selected from the Grombalia aquifer data.

Conclusion

Results of the integrated approach applied to the Grombalia case study permits to highlight the limitations of widely used techniques for nitrate source identification and apportionment in A&SAR (Table 3), and allow the selection of the best combination of tools as a function of the project goals. This would permit to maximize the benefits of the applications of these techniques, to identify the most effective approach to be used for each case study, and ensure that hydrogeochemical assessments would provide sound advices for improving the quality of local water resources and prevent future contamination episodes.

Table 3. Synthesis of the strengths and weaknesses of widely used techniques for nitrate source identification and apportionment in arid and semi-arid regions.

Technique	Goal	Main strengths	Weakness
General chemistry	To point out NO_3 contamination occurrence	Formulates a preliminary assumption of potential NO3 origin	Does not allow to separate NO_3 and salinity
Geochemical cartography and land use assessment	To highlight the spatial distribution of NO_3 concentrations	Formulates a preliminary assumption of potential NO3 origin	The outcome is highly dependent on the quality of the monitoring network (e.g. spatial distribution) Do not allow to separate NO_3 and salinity
Multivariate Statistical Analysis	To unveil common patterns among samples	Groups samples based on their dominant characteristics (particularly useful with big data sets)	Does not offer easily accessible information about the chemical composition of the samples in the clusters (groups) (Güler et al., 2002); Does not allow to separate NO_3 and salinity
Dual nitrate isotopes ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$)	In depth identification of possible NO_3 sources	Unveils sources and occurrence of denitrification processes	Possible biased result interpretation due to poor literature on sources' characteristics, and post application modifications in A&SAR; Do not allow to distinguish between sewage and manure
Socio-hydrogeological approach	In depth assessment of local application of fertilizers and manure	Cross checks with $\delta^{15}N$ and possible alternative when research funds are limited	Time consuming and requires specific training on public engagement theory
Coupled $\delta^{15}N_{NO3}$ and $\delta^{11}B$ systematics	To discriminate among manure and sewage sources of NO_3	Clear distinction of sewage and manure sources	Unsuitable for groundwater with high B geochemical background.
Bayesian mixing modelling	NO_3 source apportionment	Provides useful information on proportional contribution of each source, useful to support effective strategies for NO3 contamination reduction	Highly dependent on the value attributed to the sources.

444

445   Overall, the use of integrated approaches is fundamental for supporting sustainable groundwater

446   management, but, even in the case of well consolidated methodologies, results should be

447   interpreted taking into account regional and local features.

448

455

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