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ORIGINAL ARTICLE

Application of nitrogen and boron isotopes for tracing sources of anthropogenic contamination in Monforte-Alter do Chão aquifer system, Portugal

P. Fernandes[1] · **M. R. Carvalho**[1,2] · **M. C. Silva**[1] · **A. Rebelo**[1] · **J. Zeferino**[1]

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Abstract

The Monforte-Alter do Chão aquifer system represents one of the main water resources in Alentejo region (SW Portugal), and is located in an area of intensive agriculture and cattle breeding. The groundwater shows varied chemical composition, reflecting fast chemical changes associated with the water–rock interaction, with waters of Ca–HCO_3, Na/Ca–HCO_3, and Na–HCO_3 facies. Some of the waters have strong evidence of nitrogen contamination with nitrate concentrations above 50 mg/l. The implementation of the European Nitrates Directive and the Water Framework Directive has led to the designation of large areas as Nitrate Vulnerable Zones, for being at risk from agricultural nitrate pollution: groundwater containing or that could contain more than 50 mg/l of nitrates. Corrective measures taken to reverse the trend can only be established when the contaminant source is known. In this study, the isotopic compositions of NO_3 ($\delta^{15}N$ and $\delta^{18}O$) and B ($\delta^{11}B$) were used to identify the sources of the nitrogen contamination. Despite the occurrence of some evaporation processes, the $\delta^{15}N$ and $\delta^{18}O$ isotopes allowed to distinguish two groups of waters with different sources of nitrates, fertilizers, and soils or manure and domestic effluents. The analysis of the NO_3 source based on $\delta^{11}B$ isotope ratio pointed out contamination by pig farms effluents. However, considering the land occupation and the strong livestock activities in the area, it is possible that the adsorption of ^{11}B isotopes onto clays or the high pH of the groundwater could explain the high $\delta^{11}B$ values.

Keywords Nitrate source · N, O, and B isotopes · Monforte-Alter do Chão aquifer system

Introduction

In agriculture, the trend towards intensification of productivity during much of the past 50 years has been accompanied by the significant growth in the use of fertilizers, particularly inorganic nitrogen. Fertilizing practices that are not controlled (excessive fertilizer application and at the wrong time, among others) cause the accumulation of nitrogenous compounds in the soil, that are not absorbed during the growing season of plants and ends up being transported to the saturated zone and spread through large areas.

The presence of nitrates in groundwater in rural areas is mainly related to natural processes of nitrification, decomposition of organic matter, and anthropogenic pollution, and mainly due to widespread application of synthetic nitrogen fertilizers in agriculture. However, there are other sources of nitrate related to livestock farming and urban development that may increase the concentration of nitrate in groundwater. Livestock density also increased during most of this period, contributing to greater global burden of nitrogen on

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✉ M. R. Carvalho
mdrcarvalho@fc.ul.pt

P. Fernandes
pgfernandes@fc.ul.pt

M. C. Silva
csilva@fc.ul.pt

A. Rebelo
ar.andreia.rebelo@gmail.com

J. Zeferino
jfzeferino@fc.ul.pt

[1] Departamento de Geologia, Faculdade de Ciências, Universidade de Lisboa, Ed. C6, Campo Grande, 1749-014 Lisbon, Portugal

[2] Instituto D. Luiz, Faculdade de Ciências, Universidade de Lisboa, Ed. C1, Campo Grande, 1749-014 Lisbon, Portugal

agricultural soils resulting from animal manure, mainly cattle, pigs, poultry, and sheep. The main source of nitrogen in urban areas is mainly related to the disposal of waste water (sewage) and solid-waste disposal (landfills and dumps).

Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources aims to reduce water pollution by nitrates from agricultural sources and prevent its continued rise. The program of action can only be established when the contaminant source is known.

The implementation of the Nitrates Directive (European Commission 1991) and the Water Framework Directive (European Commission 2000) has led to the designation of large areas as vulnerable to NO_3 pollution [Nitrate Vulnerable Zones, (NVZ)]. NVZs are areas designated as being at risk from agricultural nitrate pollution: groundwater containing or that could contain (if no action is taken to reverse the trend) more than 50 mg/l of nitrates. Under the scope of the Directives, several environmental objectives were pointed out to groundwater: to prevent or limit the input of pollutants to groundwater, to protect, enhance, and restore all groundwater bodies, to implement measures to reverse any sustained and significant enrichment trend on the concentration of any pollutant introduced in groundwater due to human activities and to allow compliance for protected areas according to the respective standards and objectives.

In the NVZs, the programs defined for the reduction of NO_3 pollution are mandatory and include measures from the Codes of Good Agricultural Practices, and other measures, such as limitation of fertilizer application (mineral and organic), taking into account crop needs, all nitrogen inputs and soil nitrogen supply, and maximum amount of livestock manure to be applied (corresponding to 170 kg nitrogen/ha/year). These measures may provoke conflict with farmers if the NO_3 sources are not well identified.

The Monforte-Alter do Chão aquifer system is the geological support of the main groundwater body of SW Portugal (Fig. 1). The system has a regulatory role in water resources, minimizing the consequences of periodic droughts, assuming a strategic role in social and economic development of the region. The Monforte-Alter do Chão groundwater body is subject to multiple pressures and affected by diffuse and point-sourced contamination from agricultural activities, animal farming, and sewage treatment plants, or even lack of appropriate wastewater drainage and treatment systems.

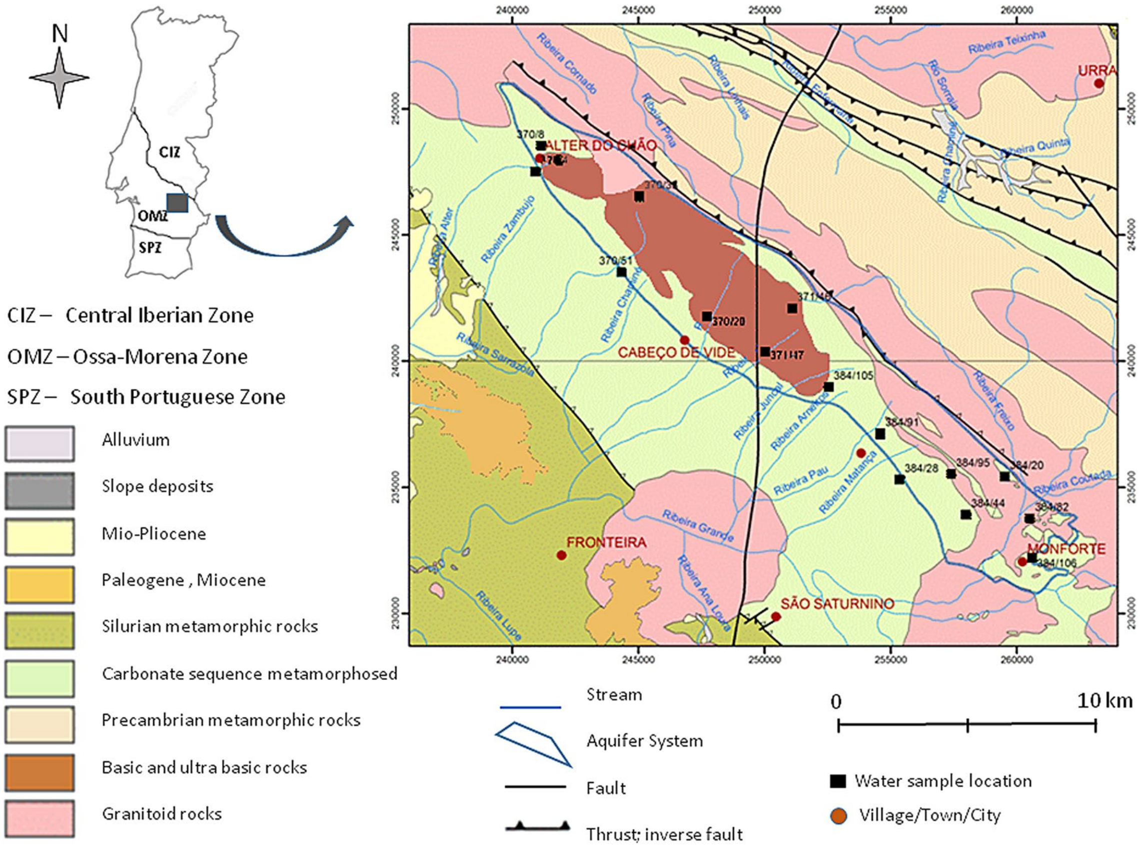


Fig. 1 Location and geologic setting of the Monforte-Alter do Chão aquifer system (Almeida et al. 2000), adapted from Gonçalves et al. (1975) and Gonçalves and Fernandes (1997); the location of the groundwater samples is indicated

The nitrate concentrations in groundwater are higher than 50 mg/l in several zones. The designation of the Monforte-Alter do Chão groundwater body as NVZ depends on the source of the nitrate contamination, as well as the application of effective mitigation.

The possibility of multiple origins and the co-existence of various biogeochemical processes and other chemical substances make it difficult to distinguish the sources of nitrate in aquifers. The literature describes several approaches for distinguishing between anthropogenic sources of nitrate in groundwater, generally coupling hydrochemistry with the isotopic data of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ in the NO_3 ion (e.g., Aravena et al. 1993; Clark and Fritz 1997; Kendall 1998; Panno et al. 2001; Xue et al. 2009; Baily et al. 2011): atmospherically deposited NO_3, synthetic (or mineral) NO_3 fertilizer, and NO_3 generated from the nitrification of ammonium (NH_4^+), derived from soils, sewage, and manure. In addition, NO_3 isotopes provide a unique tool for identifying biochemical processes, such as nitrification and denitrification, influencing NO_3 concentrations in groundwater (e.g., Bottcher et al. 1990).

Despite $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ data can distinguish several anthropogenic sources of N in groundwater, they do not distinguish manure from sewage-derived nitrates, since these sources have overlapping isotopic signatures (Kendall et al. 2007). The combined use of nitrogen and boron isotope systematics ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$, and $\delta^{11}B$) is a promising approach for the differentiation of multiple NO_3 sources (Widory et al. 2005; Xue et al. 2009) (animal manure, mineral fertilizers, and wastewater).

The main objectives of the present work are: to identify the nitrate contamination sources in the Monforte-Alter do Chão groundwater body; to identify biochemical processes that occur between soil and groundwater contributing to the accumulation or attenuation of the dissolved nitrate; to help regulators develop remediation strategies.

Study area

The Monforte-Alter do Chão aquifer system is the geological support of the main groundwater body with the same designation, supplying the villages of Alter, Alter Pedroso, Vide, Vaiamonte, and Monforte (Fig. 1), in addition to the strong agricultural and livestock farming in the region.

The aquifer system outcrop occupies a total area of 69 km^2 with an elliptical shape elongated in NW–SE direction (Fig. 1), located in the Ossa Morena Zone of the Iberian Hercynian belt, in the sector of Alter-Elvas, which lies between the faults of Alter do Chão (NE) and Juromenha (SW), (in Araújo 1995). It consists of a central aquifer surrounded by a peripheral one with hydraulic connection between them (ERHSA 2001).

The peripheral aquifer, with an approximate area of 39 km^2, is hosted by the Lower Cambrian carbonate sequence, contact-metamorphosed by mafic, and ultramafic intrusions forming an NW–SE cumulate-type structure of Ordovician age (Costa et al. 1993). In this sector, basal limestones and dolomites are followed up section by alternating shales and quartzites, conglomerates, and, finally, shales and greywackes with interbedded lenticular green rocks; sequence assigned to the lower and middle Cambrian (Delgado 1904).

The central aquifer, with an area of 30 km^2, consists of basic and ultrabasic rocks (gabbros, olivine and anorthosite gabbros, dunites, and peridotites), subjected to serpentinization—rodingitization processes, which correspond to Massif of Alter do Chão—Vide. At the South end of the system, there are various types of metamorphic rocks, including marbles, calc-silicate hornfelses, pelitic hornfelses, and a group of amphibolic metagabbros (basic hornfelses), generated by contact with granitoid magmas from the Plutonic Complex of Santa Eulalia (Lopes et al. 1998). This complex is intrusive into Monforte formations from the Proterozoic (the “Blastomilonítica” sector) and lower Paleozoic (Sector Alter-Elvas), in clear discordance with the regional structures of NW–SE orientation.

The topography of the region is mild, characterized by broad valleys and slopes that are not very pronounced. The average annual temperature of the region is around 16 °C: in summer, the average is 25 °C (July–August), but the maximum temperature may exceed 40 °C; in winter, the minimum temperature can get below 0 °C at night, but the average is 10 °C (December–January). The precipitation average is around 610–630 mm and the evapotranspiration ranges from 400 to 450 mm (Raposo and Nunes 1978). Figure 2 shows the monthly precipitation, average air temperature, average high temperature, and evapotranspiration at Portalegre meteorological station (data from IPMA 2018),

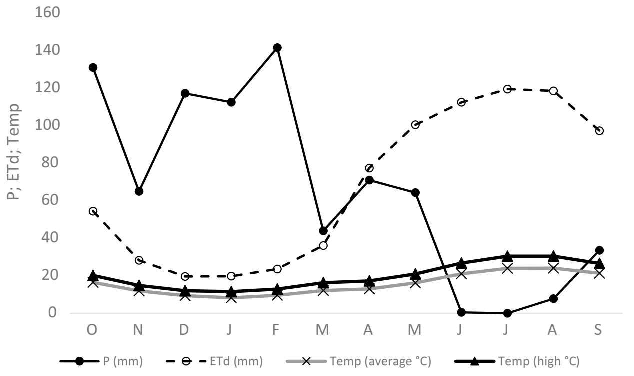


Fig. 2 Monthly precipitation, average air temperature, average high air temperature, and evapotranspiration at Portalegre meteorological station (data from IPMA 2018)

located approximately 15 km to the NE of the studied area and at elevation of 597 m a.s.l.

The aquifer system is unconfined, karstic in the limestone and dolomite formations, and of the fractured type in gabbroic rocks. The recharge is provided directly by rainfall with an annual average precipitation of 631 mm, of which 13% infiltrates, corresponding to an efficient infiltration of 82.03 l/m^2 (ERHSA 2001).

The surface and subsurface flows appear to be controlled by the geological structure, tectonics and morphology. At the regional scale, groundwater flow is towards the SW. In the extreme N and S of the system, this trend is changed, respectively, for S–SE and ESSE (Fig. 3; Fernandes 2001; Fernandes and Ribeiro 2001; Fernandes and Francés 2009). It has a natural discharge through springs, which lie essentially along the contact between the basic complex and limestones, and between limestones and the shale at the SW edge of the structure.

The carbonate formations show transmissivity between 92 and 538 m^2/day (Costa 1995) and storage coefficient range 2.15×10^{-3}–3.84×10^{-3} (Costa 1995), consequence of fractures and karst development. The mafic and ultramafic rocks have transmissivity of 282 m^2/dia. The existing data clearly indicate the presence of horizontal barriers and, consequently, the partitioning of the groundwater body.

The Monforte-Alter do Chão groundwater body is subject to multiple pressures, and affected by several point-sources of contamination from sewage treatment plants and areas where contamination is diffused, due to agricultural activities and animal farming, or even lack of appropriate waste water drainage and treatment systems. The agroforestry land use is represented in Fig. 4, through the land-use classes aggregated (COS2010) as: (1) temporary crops represents crops that mature within one or more rain seasons and are destroyed after harvesting, for example, beans and maize; (2) permanent crops are the land cultivated with crops that occupy the land for long periods and need not be replanted after each harvest; (3) permanent pastures means natural or seeded grassland that remains unploughed for many years; (4) heterogeneous crops, usually sown in rows with a variable plant; (5) forest; and others. For each soil aggregated class, the amount of nitrogen exported for the environment is estimated by Novotny and Olem (1994) and used in Portugal in environmental studies: *N* total of 5 kg/ha/year for class 1); 2.7 kg/ha/year for 2); 1.5 kg/ha/year for 3); 3.85 kg/ha/year for 4); 2 kg/ha/year for 5).

Sampling and analytical methods

Three field campaigns were carried out during autumn 2010, spring 2011, and spring 2015 for sampling waters and, for the last campaign, also soils (Carvalho et al. 2011; Rebelo 2016). A total of 16 groundwater points were sampled from the groundwater quality monitoring net of the national agency for environmental protection—Agência Portuguesa do Ambiente—APA, I.P. (Fig. 1): nine production boreholes, five wells, and the remaining springs. A total of 18 soil samples were taken, whenever possible, near the water spot (Fig. 10).

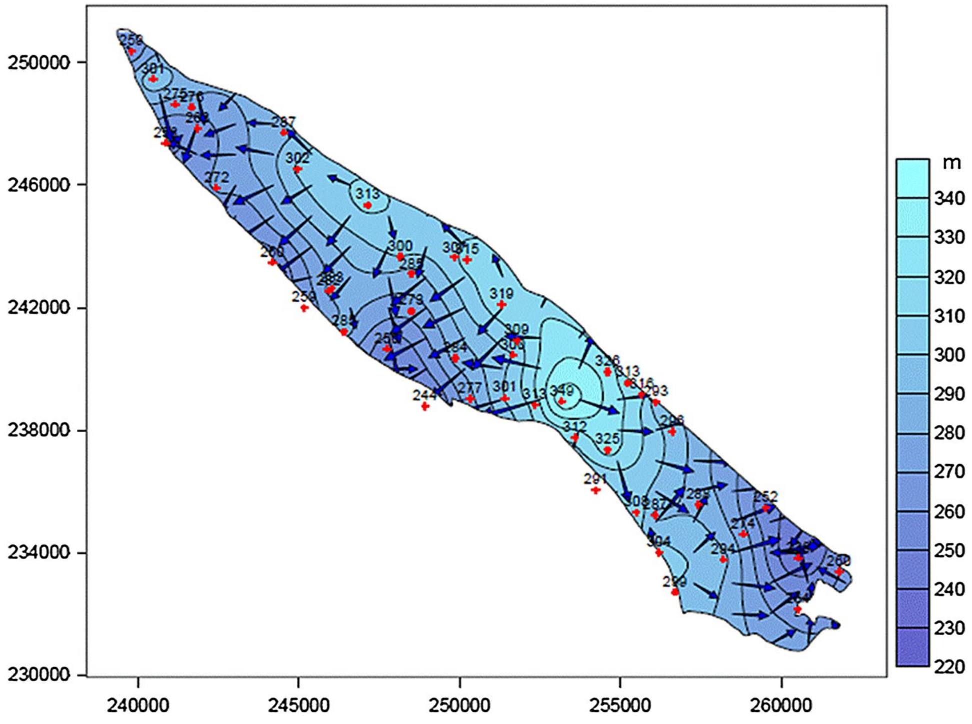


Fig. 3 Piezometric surface and groundwater flows in the Monforte-Alter do Chão aquifer system (adapted from Fernandes and Francés 2009)

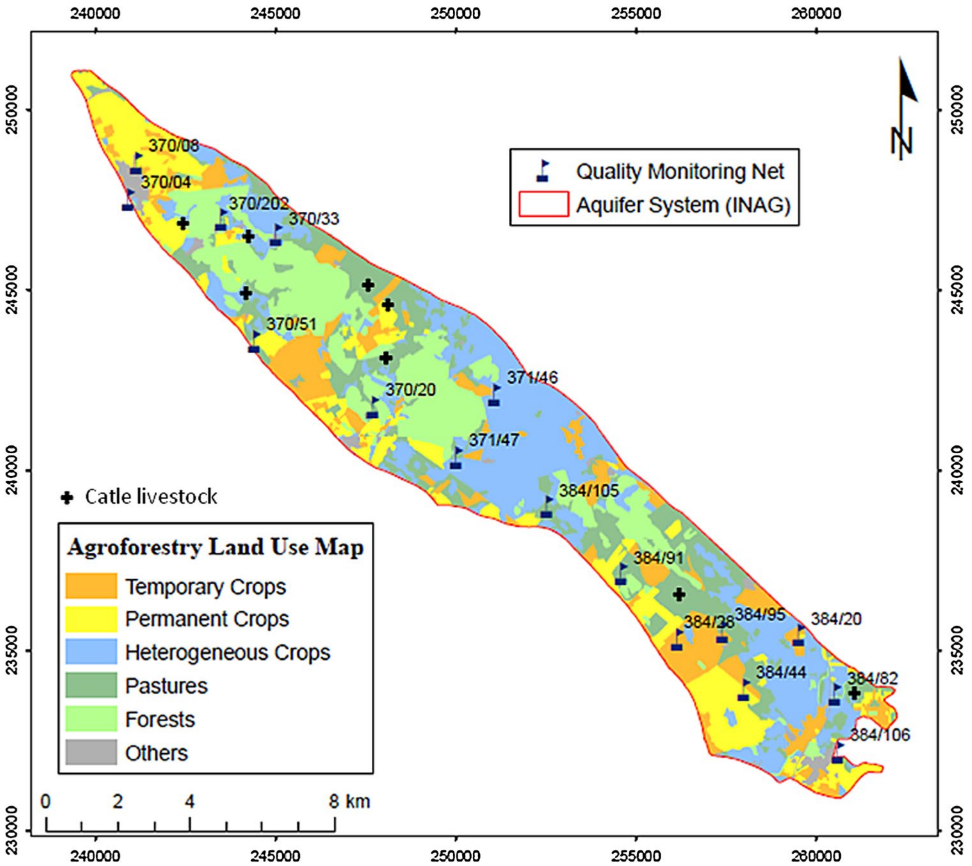


Fig. 4 Agroforestry land-use map (COS2010) for the area of the Monforte-Alter do Chão aquifer system. For each soil aggregated class, the amount of nitrogen exported for the environment is estimated by Novotny and Olem (1994) and used in Portugal in environmental studies: *N* total of 5 kg/ha/year for class (1); 2.7 kg/ha/year for (2); 1.5 kg/ha/year for (3); 3.85 kg/ha/year for (4); 2 kg/ha/year for (5); location of the main cattle livestock

Field investigations of the groundwater included the in situ measurements which were: temperature (T), pH, electric conductivity (EC), and redox potential (Eh), with portable WTW equipments. For chemical analysis of cations, the water samples were readily filtered with a 0.45 µm pore diameter filter and acidified with ultra-pure nitric acid. Samples for anion analysis were kept unacidified. The samples for isotopic analysis were taken in polyethylene bottles with double cap, of 150 ml for δ^{18}O and δ^{2}H in H_2O, 1 l for δ^{18}O and δ^{15}N in NO_3, and 1 l for δ^{11}B.

The samples collected during the first campaign were analyzed (cations and anions) at the laboratory LPQ—Laboratório Pró-Qualidade (Portugal), by potentiometry and ion chromatography. The alkalinity was analyzed by H_2SO_4 (0.05 M) titration and the free CO_2 by NaOH (1/44 mol), both at Faculdade de Ciências of the Universidade de Lisboa. The dissolved metals were analyzed by ICP-MS and ICP-OES at Activation Laboratories Ltd. (Canada). The δ^{2}H and δ^{18}O measurements (vs V-SMOW, Vienna-Standard Mean Ocean Water) were performed by mass spectrometry, at the SIIAF—Stable Isotopes and Instrumental Analysis Facility (Portugal). The nitrate extraction and δ^{18}O and δ^{15}N determination were made in frozen samples shipped to the Environmental Isotope Laboratory in the University of Waterloo (Canada), and analyzed with a continuous flow isotope-ratio mass spectrometer, with precision of 0.5‰. The samples from the second campaign were analyzed only for anions and metals at laboratory LPQ–Laboratório Pró-Qualidade (Portugal), by potentiometry, ion chromatography, and atomic absorption spectrometry (AAE). The isotopic ratio of δ^{11}B in water was measured by ICP—SFMS techniques, precision about 1%, at ASL Environment Laboratory (Sweden). In the third campaign, the main interest was the characterization of the soils; only a few groundwater points were sampled for analysis and were analyzed at the Activation Laboratories Ltd. (Canada) by ion chromatography and ICP-MS.

The crop residues of soils were removed before collection. The soil samples were air-dried, homogenized, and analyzed at Faculdade de Ciências of the Universidade de Lisboa. Each sample was quartered and 100 g of material were processed for grain size using a sieve of 63 µm. The finer material was then dried at 60 °C in sand bath and the coarse material was oven dried at 100 °C. The distribution of particle size in the soil fraction < 63 µm was measured using the laser diffraction method by the Malvern Particle Size Analyzer MS2000. To characterize OM quality in soil, i.e., the percentages of labile and refractory OM, the stepwise thermogravimetric procedure (STG) of Kristensen (1990) was applied. According to this method, these OM fractions are defined as the percentage weight losses after ignition at 280 and 520 °C, respectively. Concentrations of total carbon and total nitrogen were determined by elemental analysis.

Results

Groundwater chemical composition

Groundwater composition displays different chemical facies in response to the interaction with how different lithologies of the aquifer system (e.g., Carvalho et al. 2011; Rebelo 2016). The major characteristics and constituents of the groundwater are presented in Tables 1, 2, and 3. Groundwaters flowing within the Cambrian limestones are Ca–HCO_3-type waters, with neutral-to-slightly basic pH, and medium-to-high mineralization. The groundwaters with Ca/Mg–HCO_3 composition (Fig. 5) are neutral and of medium mineralization. This type of water is associated with aquifers developed in gabbroic rocks and some springs are located at the geologic contact with metamorphosed limestones. In this last case, the Mg present can be attributed to the presence of dolomite found in the limestone rocks. One water (370/33) has Ca–Cl/HCO_3 composition (Fig. 5), probably as a consequence of strong contamination and evaporation. The Mg–HCO_3 type waters include most of the groundwaters discharging from the gabbros and serpentinized dunites, suggesting that water chemistry is strongly host rock dependent. Near Monforte and along the northern edge of the aquifer system, there is an enrichment in chloride content, probably associated with anthropogenic urban areas and pasture, although we cannot exclude the possibility of mixing with groundwater from other formations.

The distribution of NO_3 concentrations in groundwater, in autumn’10, spring’11, and spring’15 is reported in Figs. 6, 7, and 8, respectively. High concentrations, often exceeding 50 mg/l, are observed in groundwater during autumn’10, and it lower ones during spring’11. These values can reflect dilution with rain water at the wet season. The nitrate concentrations are much lower in the samples from the third campaigns, probably because the corrective measures and good farming practices had already been applied. In the first sampling, only sample 371/47 has nitrogen as NO_2 and NH_4 (0.58 and 0.12 mg/l, respectively,, in Table 1); the samples 384/44 and 384/105 have 0.4 mg/l of dissolved NO_2 (Table 1). In the second sampling, the samples 370/51, 371/47, 384/44, and 386/106 have detectable concentration of NH_4 (Table 2).

Groundwater isotopic composition

Groundwater from Monforte-Alter Chão aquifer system, on average, is depleted in heavier isotopes relatively to the

Table 1 Chemical composition of the groundwater sampled during autumn’10; *EC* electric conductivity, *Alk* alkalinity as $CaCO_3$, *n.d.* not determined

Sample	pH/Temp. (°C)	EC (µS/cm)	Eh (mV)	Alk. (mg/l)	Ca (mg/l)	Na (mg/l)	K (mg/l)	Mg (mg/l)	Cl (mg/l)	SO_4 (mg/l)	NO_3 (mg/l)	NO_2 (mg/l)	NH_4 (mg/l)	Fe (µg/l)	Mn (µg/l)
370/04	6.99/20.3	711	+243	340	107.0	16.8	1.24	26.2	19	<10	21	<0.02	0.03	<10	0.2
370/20	7.58/19.3	665	+241	375	41.5	9.9	0.51	70.1	14	<10	12	<0.02	0.02	10	0.1
384/20	7.24/26.8	1153	+218	216	98.3	26.4	7.32	22.3	68	49	22	<0.02	<0.02	60	14.9
384/28	6.97/19.3	640	+279	303	99.6	12.9	1.12	11.9	14	15	37			40	8.1
370/202	7.25/19.3	663	+134	226	78.0	23.2	0.60	35.1	36	<10	16	<0.02	<0.02	40	3.2
384/44	7.44/19.4	656	+204	368	117.0	17.3	3.00	12.3	21	23	50	0.04	<0.02	20	57.8
371/47	7.41/20.7	744	+213	226	27.5	8.38	0.71	72.0	18	<10	<10	0.58	0.12	30	1.3
370/51	7.15/20.1	558	+251	242	103.0	15.4	1.18	20.3	20	15	33	<0.02	<0.02	20	0.5
384/82	7.12/20.7	687	+235	271	38.8	28.7	1.65	12.0	34	<10	21	<0.02	<0.02	<10	0.4
384/91	6.99/24.8	424	+237	1295	82.0	17.6	1.02	24.9	16	<10	22	<0.02	<0.02	40	22.5
384/95	7.43/24.6	656	+238	260	73.5	23.4	2.68	22.6	14	29	34	<0.02	<0.02	30	0.3
384/105	7.34/19.1	555	+252	246	73.9	15.2	1.12	30.0	25	19	24	0.4	<0.02	30	0.2
384/106	7.82/19.9	579	+221	264	51.1	46.2	3.68	36.3	34	29	66	<0.02	<0.02	30	0.2
370/33	6.91/20.9	675	+257	226	108.0	34.6	4.52	59.2	130	68	96	<0.02	n.d.	10	0.1

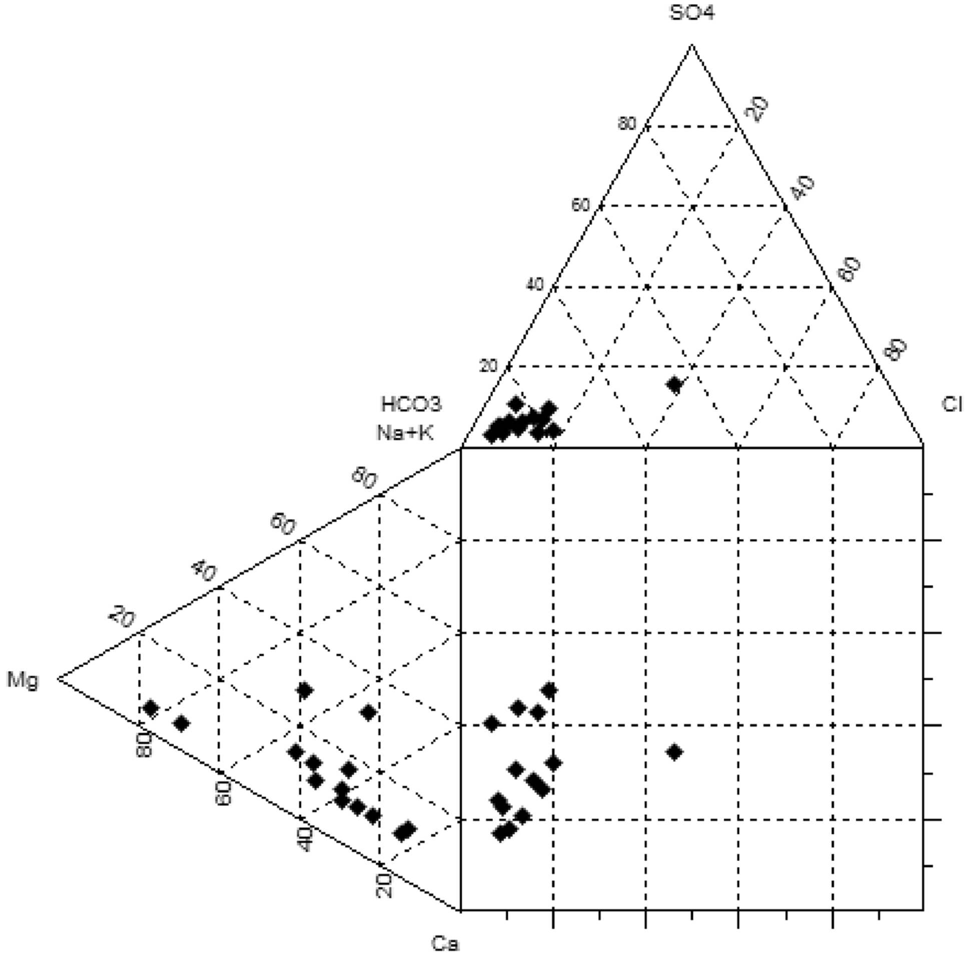
Table 2 Chemical composition of the groundwater sampled during spring'11; *EC* electric conductivity; *Alk* alkalinity—alkalinity as $CaCO_3$; *n.d.* not determined

Sample	pH/Temp. (°C)	EC (µS/cm)	Eh (mV)	Alk. (mg/l)	Cl (mg/l)	SO_4 (mg/l)	NO_3 (mg/l)	NO_2 (mg/l)	NH_4 (mg/l)	Fe (µg/l)	Mn (µg/l)
370/04	7.84/18.8	709	+273	288	22	35	32	< 0.02	< 0.02	< 50	< 15
370/08	7.20/15.8	552	+254	385	15	14	15	< 0.02	< 0.02	< 50	< 15
370/20	7.58/17.7	690	n.d.	427	14	13	13	< 0.02	< 0.02	< 50	< 15
370/33	6.71/24.0	1398	+208	248	170	83	28	< 0.02	< 0.02	< 50	< 15
370/51	6.84/18.2	607	+209	257	17	13	28	< 0.02	0.06	< 50	< 15
370/202	7.99/14.3	428	− 95	211	25	37	< 10	< 0.02	< 0.02	820	200
371/47	8.40/29.4	796	+173	426	16	10	< 10	0.11	0.10	200	27
384/20	6.78/22.4	1131	+200	325	77	55	46	< 0.02	< 0.02	< 50	< 15
384/28	6.96/18.7	1131	+440	212	11	12	32	< 0.02	< 0.02	< 50	< 15
384/44	6.98/15.3	582	+272	242	17	25	46	< 0.02	0.03	< 50	< 15
384/82	6.52/14.2	340	+287	104	24	19	40	< 0.02	< 0.02	< 50	< 15
384/91	7.48/27.5	682	+191	271	14	28	36	< 0.02	< 0.02	< 50	< 15
384/95	7.12/15.5	455	+380	220	12	13	20	< 0.02	< 0.02	< 50	< 15
384/105	7.14/16.0	560	n.d.	292	19	19	24	< 0.02	< 0.02	< 50	< 15
384/106	7.56/12.8	591	+183	251	51	31	46	< 0.02	0.03	280	< 15

Table 3 Chemical composition of the groundwater sampled during spring'15; *Alk* alkalinity as $CaCO_3$; *n.d.* not determined

Sample	pH/Temp. (°C)	EC (µS/cm)	Alk. (mg/l)	Ca (mg/l)	Na (mg/l)	K (mg/l)	Mg (mg/l)	Cl (mg/l)	SO_4 (mg/l)	NO_3 (mg/l)	B (µg/l)	Fe (µg/l)	Mn (µg/l)
370/04	6.82/21.7	811	307	103.0	28.8	3.46	35.2	38	36	11	46	< 10	< 0.1
370/08	7.01/18.5	667	307	97.0	14.9	1.55	28.4	17	18	3	8	20	< 0.1
370/20	7.47/19.8	815	419	48.2	11.0	0.45	83.2	21	16	4	12	20	0.4
371/47	8.75/33.5	1366	n.d.	9.7	18.4	11.80	24.2	67	40	< 1	26	30	156
384/20	6.93/20.8	902	256	120.0	28.5	12.9	27.4	73	65	9	41	20	11.3
384/82	7.14/25.9	356	87	32.0	23.1	1.51	9.96	28	12	8	13	< 10	< 0.1
384/91	7.18/23.3	519	206	79.0	13.1	3.08	14.8	14	16	6	6	< 10	< 0.1
ZI	6.82/19.5	720	338	89.2	13.6	0.85	25.2	18	24	4	9	< 10	< 0.1

Fig. 5 Groundwater samples represented in a Durov diagram (% of meq/l) show Ca/Mg–HCO_3 and Mg–HCO_3 types and one Ca–Cl/HCO_3 composition



international standard V-SMOW (Table 4). The $\delta^{18}O$ ratio varies between −5.24 and −3.90‰; the $\delta^{2}H$ ratio is between −29.6 and −22.3‰, ranging 1.34 and 7.3‰, respectively. With respect to the excess of deuterium, the values obtained are higher than the value of the excess deuterium presented by global meteoric water line (GMWL) defined by Craig 1961 (Fig. 9), and range between 8.9 and 14.5‰. The precipitation collected at Portalegre meteorological station has long-term-weighted isotopic mean values of $\delta^{18}O = -5.21$‰ and $\delta^{2}H = -34.6$‰ (IAEA/WMO 2017). The local meteoric water line (LMWL) obtained with monthly data (Fig. 9) is represented by the equation $\delta^{2}H = 5.35\delta^{18}O - 2.996$ (with $R^2 = 0.93$). The monthly isotopic composition varies strongly from winter to summer, $\delta^{18}O = -6.24$‰ and $\delta^{2}H = -36.8$‰ in November, and $\delta^{18}O = -2.37$‰ and $\delta^{2}H = -17.2$‰ in August. The monthly d-excess and air temperature (IAEA/WMO 2017) shows significant seasonal changes in the interplay between subcloud evaporation and moisture recycling, manifesting an inverse temperature effect.

The groundwater isotopic compositions show a slight deviation from LMWL, as can be seen in Fig. 9, with high d-excess and shallower slope, and approach the regression line $\delta^{2}H = 4.82\delta^{18}O - 3.50$‰, with a correlation coefficient of 0.8. The lower slope and intercept (when compared with GMWL) are typical for a warm dry climate, where subcloud evaporation of raindrops is experienced. These results are in accordance with the climate of Monforte-Alter do Chão region.

The variation measured on the isotopic composition of $\delta^{18}O_{NO3}$ and $\delta^{15}N_{NO3}$ is important, ranging from +9.2‰ to +15.37‰ and from +2.85‰ to +16.73‰, respectively, in autumn'10; from +5.65‰ to +10.73‰ and +2.94‰ to +16.18‰ in spring'11. Greater variation is observed in $\delta^{18}O$ values than $\delta^{15}N$, from one season to another and the samples of spring'11 are more depleted in ^{15}N. The $\delta^{15}N_{NO3}$ values are higher in the margins of the aquifer systems close to the villages of Monforte and Alter do Chão.

The isotope ratio of $\delta^{11}B$ show values between 28.5 and 41.0‰, and the higher values are spatially related with the higher $\delta^{15}N_{NO3}$.

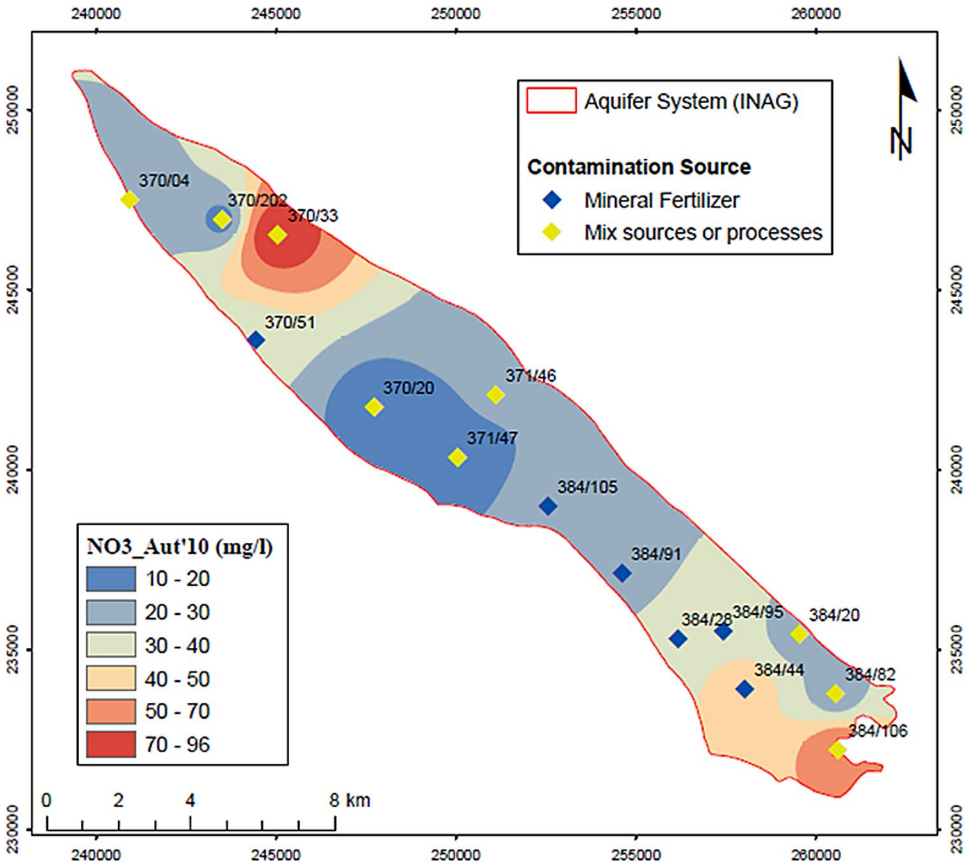


Fig. 6 Distribution of the dissolved NO_3 (mg/l) in groundwater for the autumn 2010 campaign. The possible source of dissolved nitrates based on isotopic data is represented (see text for explanation)

Soils

The possible correlation between NO_3 concentration in groundwater and potentially relevant soil characteristics, such as organic carbon content (that determines soil acidity) and clay content, is well known (Bohlke 2002). In general, the nitrates in groundwater increase with increasing soil organic carbon and decreasing soil pH; soils with clay contents greater than 20% seem to retain more NO_3 than coarse-grained soils, possibly by inhibiting leaching and recharge (Sacchi 2013). The organic matter and clay contents in the soil from the area of the Monforte-Alter do Chão aquifer system are examined and are represented in Table 5. The organic matter in soils ranges from 1.45 to 17.41% (Fig. 10): the lower values were determined in the SE part of the aquifer system; the higher values are in the central part of the system and close to the village of Alter do Chão, at the NW. The proportion of coarse-grained soils (> 63 µm) varies from 22.53 to 91.48%, with finer soils (silt and clay) more frequent in the SE part of the aquifer system (Fig. 11).

Nitrate sources and transformations

$\delta^{15}N$ and $\delta^{18}O$ in dissolved nitrate

Under ideal circumstances, the stable isotopes of nitrogen are good indicators of the origin or source of pollution (see general review by Kendall 1998). The value of $\delta^{15}N$ of nitrogen compounds in saturated and unsaturated zones is controlled mainly by variations in the source and sinks, and chemical, physical, and biological changes in soil and aquifers. The nitrification of ammonia and/or organic fertilizer-N, denitrification, phenomena of precipitation, and organic waste can cause a wide range of $\delta^{15}N$ value.

The composition of natural media in $\delta^{15}N$ varies between − 20 and + 30‰ (Kendall 1998; Kendall and McDonnell 1998). The $\delta^{15}N$ values of atmospheric NO_3^- and NH_4^+ are usually in the range of − 15 to + 15‰, relative to atmospheric N_2 (0‰) (Kendall et al. 2007). Many plants fix nitrogen and microorganisms incorporate it into the soil with $\delta^{15}N$ from + 3 to + 8‰ (Kendall

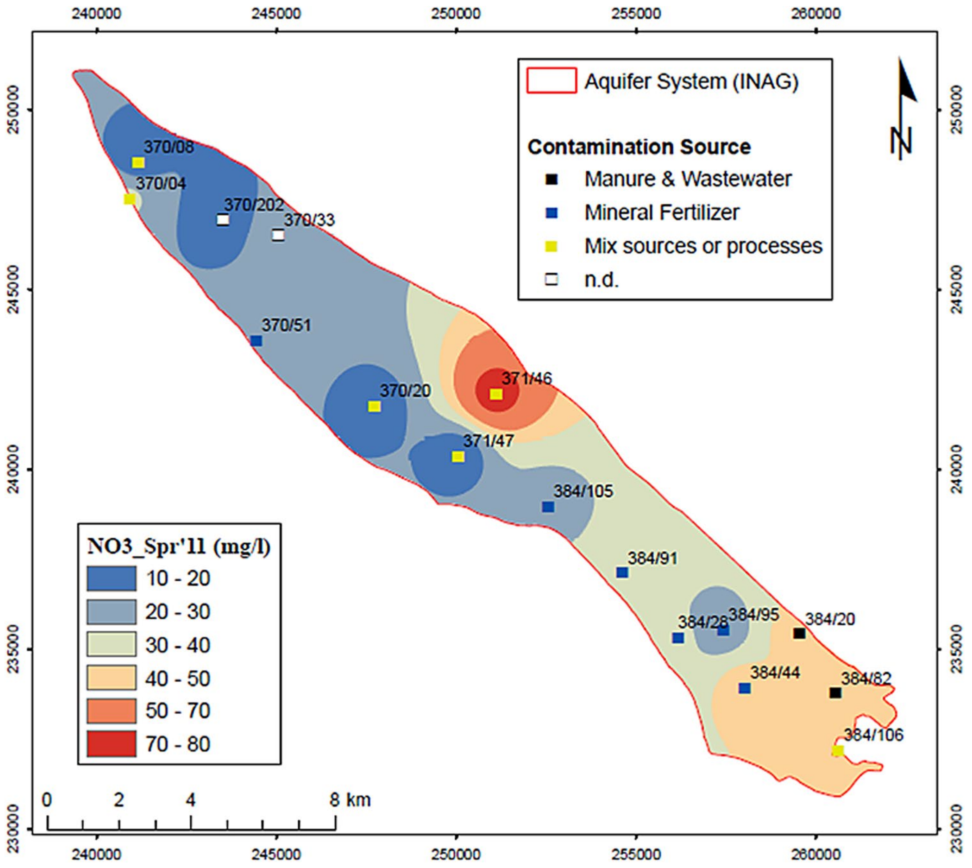


Fig. 7 Distribution of the dissolved NO_3 (mg/l) in groundwater in spring 2011 campaign. The possible source of dissolved nitrates based on isotopic data is represented (see text for explanation)

et al. 2007; Aravena et al. 2010). The nitrogen of animal manure is hydrolyzed to NH_3 and converted to NH_4^+ in the soil and, finally, to NO_3^-, with $\delta^{15}N$ between + 8 and + 20‰ (Kreitler 1979; Kendall 1998; Kendall and McDonnell 1998; Vitòria et al. 2004). This range is due to the local environmental conditions, which influence the volatilization of NH_3 and the ^{15}N enrichment in the final NO_3^- product. Nitrogen derived from wastewater is isotopically enriched with ^{15}N and $\delta^{15}N$ values which range from + 5 to + 20‰ (Curt et al. 2004; Aravena et al. 2010; Vane et al. 2010). Other nitrogen source is mineral fertilizers, with NO_3^- and NH_4^+ synthesized through industrial fixation of atmospheric N with $\delta^{15}N$ signature from − 4 to + 8‰ (Michalski et al. 2015).

The $\delta^{15}N_{NO3}$ content in Monforte-Alter do Chão groundwater body ranges from + 2.85 to + 16.73‰ (mean of + 8.89‰) in autumn, and from + 2.94 to + 16.18‰ (mean of + 7.17‰) in spring, which showed that nitrate in autumn was enriched in ^{15}N relative to that in spring (Figs. 6, 7, 8), except for samples 370/04, 370/20, 370/51, and 370/46 all located in the NW part of the aquifer system. There was no clear correlation between $\delta^{15}N$ and NO_3^- concentration (Fig. 12) and $\delta^{15}N$ vs Log NO_3^-; we find high dissolved NO_3 values with low and high $\delta^{15}N$ ratios. Ammonia and NO_2 concentrations in almost all samples are less than 0.02 mg/l, indicating that fractionation caused by incomplete nitrification is not affecting $\delta^{15}N_{NO3}$ values.

Based on the $\delta^{15}N$ value, it is possible to identify three groups of samples with distinct origins of nitrogen (Fig. 13): one with $\delta^{15}N$ lower than + 5‰; other with $\delta^{15}N$ between + 5 and + 8‰; the third has water enriched in ^{15}N, with $\delta^{15}N$ higher than + 8‰. The first group (samples 370/51, 384/105, 384/106, 384/28, and 384/44) is related with nitrogen from soil, chemical fertilizers, or mixing of contaminants; the second group (samples 370/04, 370/20, 384/91, and 384/95) can result of mixing processes such as fertilizers, organic N from soil and manure, and wastewater; the third group (samples 370/202, 370/33, 370/46, 370/47, 384/20, and 384/82) comes from domestic effluent or animal manure; the 370/51 has $\delta^{15}N$ typically of NH_4 fertilizers. From the spring'10 to the spring'15, the samples became enriched in ^{15}N, but the dissolved NO_3 is lower in groundwater. The water enrichment in N heavy isotopes means that there was greater contribution of nitrogen from organic fertilizers or septic tanks or denitrification processes occurred (Kendall 1998). Depletion in heavy isotopes means lesser contribution of this source or higher input from N of fertilizer or soil, showing the influence of agriculture on groundwater. However, the spring'15 samples contain less dissolved NO_3 and may indicate that some measures to reduce nitrogen loads

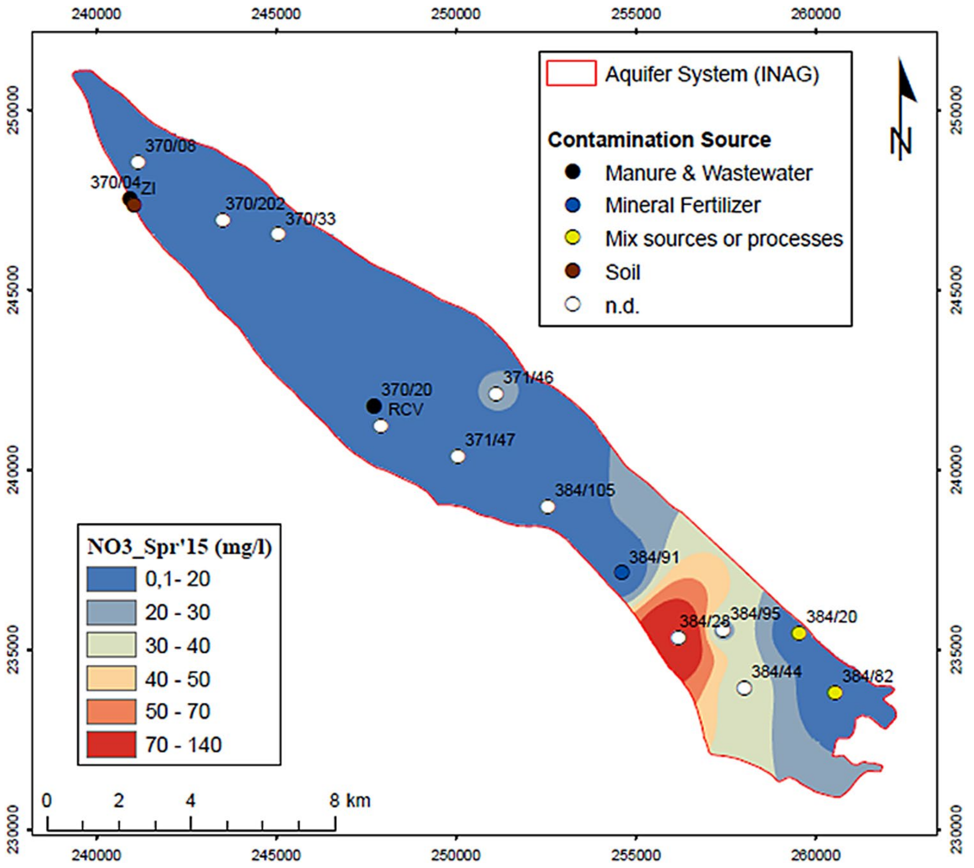


Fig. 8 Distribution of the dissolved NO_3 (mg/l) in groundwater for the spring 2015 campaign. The possible source of dissolved nitrates based on isotopic data is represented (see text for explanation)

Table 4 Isotopic composition of the groundwater from Monforte-Alter do Chão aquifer system; *n.d.* not determined

Water sample	Autumn'10				Spring'11				Spring'15	
	$\delta^{18}O_{H_2O}$	$\delta^{2}H_{H_2O}$	$\delta^{15}N_{NO_3}$	$\delta^{18}O_{NO_3}$	$\delta^{15}N_{NO_3}$	$\delta^{18}O_{NO_3}$	δ^{11}B	δ^{11}B SD^{2}	$\delta^{15}N_{NO_3}$	$\delta^{18}O_{NO_3}$
370/04	−4.91	−27.5	6.40	n.d.	9.37	10.73	37.6	3.4	10.75	4.19
370/08	n.d.	n.d.	n.d.	n.d.	7.14	n.d.	39.2	4.0	n.d.	n.d.
370/20	−4.78	−26.4	6.27	n.d.	7.82	9.02	31.7	2.2	8.19	5.33
370/202	−4.91	−25.8	14.59	13.73	n.d.	n.d.	44.0	2.6	n.d.	n.d.
370/33	n.d.	n.d.	16.73	10.51	n.d.	n.d.	39.4	1.8	n.d.	n.d.
370/51	−4.70	−25.0	2.85	9.20	4.34	8.03	n.d.	n.d.	n.d.	n.d.
371/46	n.d.	n.d.	14.59	13.73	16.18	10.29	n.d.	n.d.	n.d.	n.d.
371/47	−3.90	−22.3	12.19	15.37	9.52	n.d.	37.3	1.2	n.d.	n.d.
384/20	−5.15	−28.7	13.91	n.d.	12.26	7.69	39.5	1.4	19.38	12.41
384/28	−5.24	−27.4	3.66	9.13	3.51	6.23	n.d.	n.d.	n.d.	n.d.
384/44	−5.15	−29.3	4.93	13.06	3.84	5.65	32.0	6.5	n.d.	n.d.
384/82	−5.13	−29.6	14.17	13.30	8.68	5.85	n.d.	n.d.	11.09	10.22
384/91	−5.11	−28.2	5.19	n.d.	2.94	7.63	33.9	2.8	3.44	5.20
384/95	−5.23	−28.2	5.59	n.d.	3.89	9.32	38.8	3.2	n.d.	n.d.
384/105	−4.44	−25.6	3.86	10.36	3.56	6.73	28.5	4.6	n.d.	n.d.
384/106	−4.95	−28.4	8.74	9.31	7.35	10.21	41.0	4.2	n.d.	n.d.
ZI	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.20	4.43

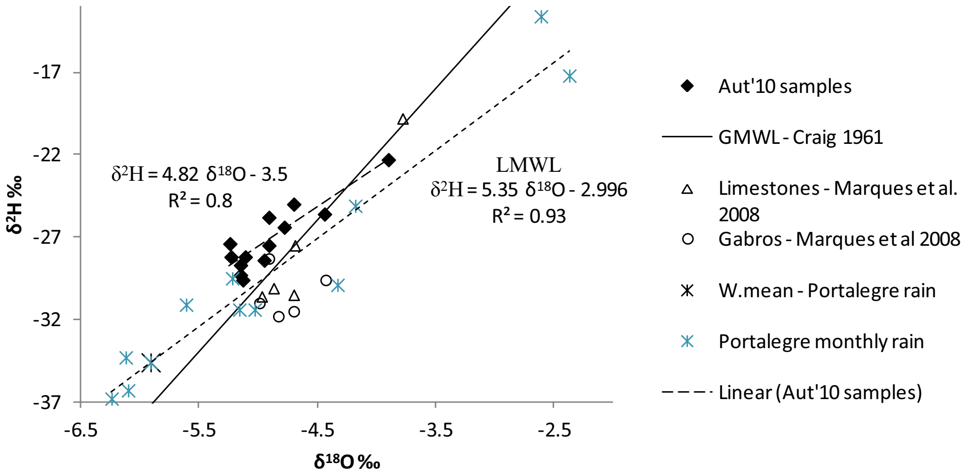


Fig. 9 Isotopic composition of δ^{18}O and δ^{2}H from Monforte-Alter do Chão aquifer system and its location in relation to GMWL (Craig 1961) and LMWL (Portalegre meteorological station, IAEA GNIP/WISER data set); isotopic data obtained by Marques et al. (2008) for groundwaters are represented

as synthetic fertilizers were used. There is no clear relationship between the nitrogen isotopes and the groundwater flow directions.

Joint analysis of isotopes δ^{18}O and δ^{15}N of nitrate allows a better interpretation of the sources and transformations of nitrate in groundwater and (Kreitler 1979; Heaton 1986; Clark and Fritz 1997; Kendall 1998), clarifying dubious situations, such as the presence of (Fig. 14): nitrate derived from nitrification processes in soils; denitrification processes; nitrate originating from organic fertilizers and/or septic tanks; nitrate-containing synthetic fertilizers; nitrate from atmospheric deposition. Compared with the δ^{15}N values, the $\delta^{18}O_{NO3}$ varies strongly from one campaign to another (Fig. 13), namely for the samples from the SE part of the aquifer system. Here is where the soil is more clayey and contains more organic matter. It has been shown that nitrate whose N is of atmospheric origin has greatly positive $\delta^{18}O_{NO3}$ (between + 30 to + 80‰; Kendall 1998); nitrate from synthetic fertilizers has $\delta^{18}O_{NO3}$ values between + 17 and + 25‰ (Xue et al. 2009; Aravena et al. 2010). The $\delta^{18}O_{NO3}$ values in nitrate derived from nitrification processes in soils and nitrate originating from septic tanks and organic fertilizers are similar, but different ranges are pointed by different studies. According to Kendall (1998) and Kendall et al. (2007), the $\delta^{18}O_{NO3}$ values of nitrified synthetic fertilizers are typically lower than + 15‰, depending on the groundwater isotopic composition; Puig et al. (2017) report values between 3.4 and 4.6‰; Sacchi (2013) assumed a $\delta^{18}O_{NO3}$ value of + 9‰ for the top limit of nitrogen from synthetic fertilizers and septic tanks and organic fertilizers. The $\delta^{18}O_{NO3}$ values proposed by Puig et al. (2017) and Sacchi (2013) seem too low for the Monforte-Alter do Chão groundwaters (Fig. 14).

The $\delta^{18}O_{NO3}$ in groundwater from the Monforte-Alter do Chão aquifer system ranged from + 9.13 to + 15.37‰ (mean of + 11.77‰) in autumn, and from + 5.65 to + 10.73‰ (mean of + 8.12‰) in spring ('11), except for samples 384/106 and 384/20. The isotopic compositions of the groundwater nitrate were plotted in the diagram $\delta^{18}O_{NO3}$ vs $\delta^{15}N_{NO3}$ (Fig. 14) and several sources are in evidence. No sample falls within the field of pure synthetic fertilizers. Considering the upper limit of + 15‰ proposed by Kendall et al. (2007) for $\delta^{18}O_{NO3}$ from synthetic fertilizers and manure or wastewater, it can be seen that: samples 384/20 and 384/82 have N from manure or wastewater contamination (located around the Monforte village); the samples 370/51, 384/105, 384/28, 384/44, and 384/91 fall in the field of NH_4 fertilizer or soil; all the others fall into the overlapping field of nitrate with N source in soil, organic fertilizers

Table 5 Characteristics of the soil in the area of Monforte-Alter do Chão aquifer system, sampled in spring'15

Soil sample	O.M. (%)	Particles < 63 µm (%)	Silt size (%)	Clay size (%)
N1	7.07	14.00	13.04	0.96
N2	3.47	22.07	19.86	2.22
N3	4.87	25.52	21.63	3.89
N4	1.45	8.52	7.59	0.92
N5	2.97	43.16	39.66	3.50
N6	3.65	48.50	43.71	4.80
N7	5.99	77.47	69.42	8.04
N8	4.71	43.16	38.29	4.87
N9	10.84	26.27	24.81	1.46
N10	12.84	46.96	42.64	4.32
N11	5.93	32.79	29.54	3.26
N12	7.21	49.12	41.47	7.65
N13	8.70	46.21	40.79	5.42
N14	4.47	19.16	17.03	2.12
N15	5.62	49.46	42.65	6.81
N16	6.79	50.83	43.92	6.91
N17	17.41	23.19	20.91	2.28
N18	5.04	48.41	44.00	4.41

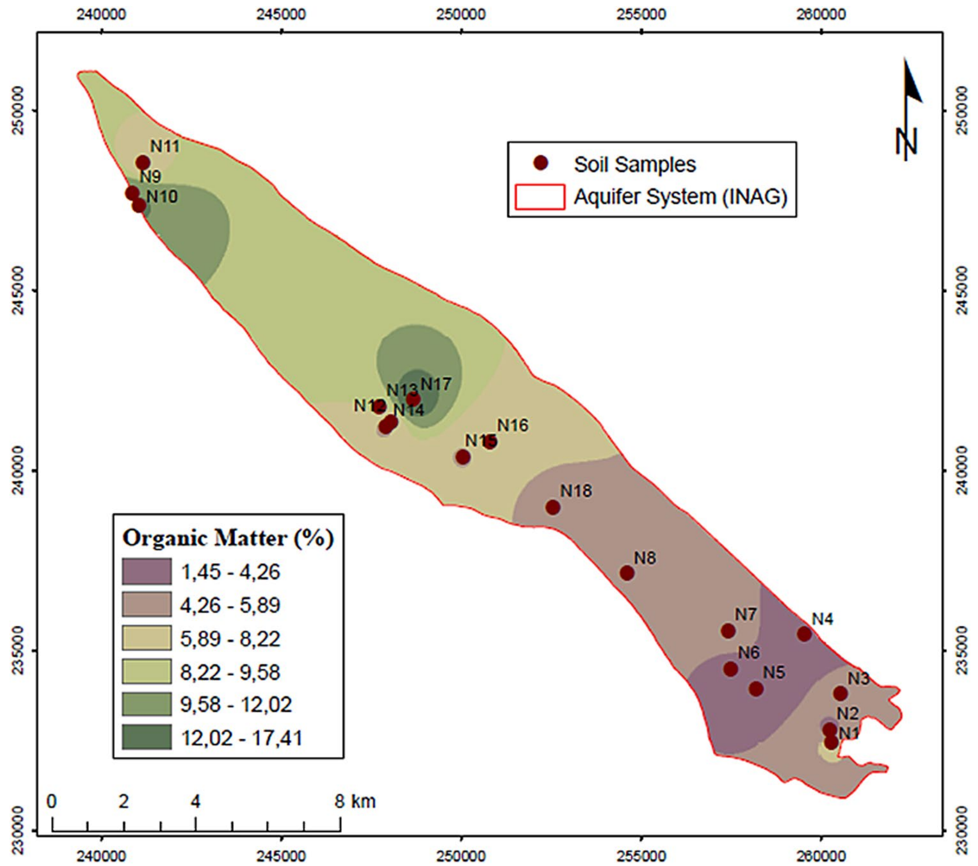


Fig. 10 Organic matter distribution in soils from the area of Monforte-Alter do Chão aquifer system

(or effluents from livestock activity) or septic tanks. The samples collected in autumn' 10 are enriched in $\delta^{18}O_{NO3}$ relative to samples from spring' 11; this can reflect strong evaporation in summer. During nitrification, the $\delta^{18}O_{NO3}$ ratio changes to lower values because of the incorporation or the exchange with $\delta^{18}O_{H2O}$ of groundwater, but, as it was mentioned above, in the Monforte-Alter do Chão region occurs subcloud evaporation of raindrops during the dry season and the groundwater has heavier isotope composition.

Some processes occurring in the soil and the vadose zone may enrich nitrate in ^{18}O: denitrification (Clark and Fritz 1997; Kendall 1998 and references therein); evaporation of soil/water/groundwater (Craig et al. 1963; Faure and Mensing 2005); aerobic soil respiration (Lane and Dole 1956; Guy et al. 1989). To observe the effect of evaporation on the $\delta^{15}N$ values, the groundwater samples were projected in a diagram $\delta^{15}N$ vs dissolved Cl (Fig. 15a), and no correlation was obtained.

The denitrification process, which is the reduction of NO_3 to gaseous N_2, requires denitrifying bacteria, low-oxygen conditions, and an electron donor. A plot $\delta^{15}N_{NO3}$ vs Eh (Fig. 15b) does not show any relation in the sampled waters, although the sample 371/46 has the lowest Eh value and highest $\delta^{15}N_{NO3}$ ratio. This singularity may be due to local contamination by manure; the oxidation of the organic matter causes reduction of Eh and NO_3 which presents enrichment in ^{15}N.

During denitrification, both $\delta^{18}O_{NO3}$ and $\delta^{15}N_{NO3}$ increase in the ratio 2:1 (Kendall 1998; Fig. 14). As denitrification progresses, residual nitrate becomes increasingly isotopically enriched, so that downgradient samples low in nitrate often provide isotopic evidence for denitrification. Any inverse correlation was identified in the collected samples between NO_3 concentration and ^{15}N enrichment, and no spatial relation was detected between the soil characteristics (organic matter content or clay content) and dissolved NO_3^- or $\delta^{15}N_{NO3}$.

$^{11}B/^{10}B$ isotope ratios

The highly soluble nature of boron (B) in the form of boric acid or borate ion favours its release to the environment (Vengosh 1998). Boron has two stable isotopes, ^{10}B and ^{11}B (relative abundance of 80.2 and 19.8%, respectively). The low atomic mass of boron induces a large percental difference in mass between the isotopes, which leads to a significant isotopic fractionation in nature. In groundwater, the $\delta^{11}B$ isotopic composition is controlled by the aquifer matrix, while in impacted environments may be influenced also by anthropogenic sources. The previous studies of B

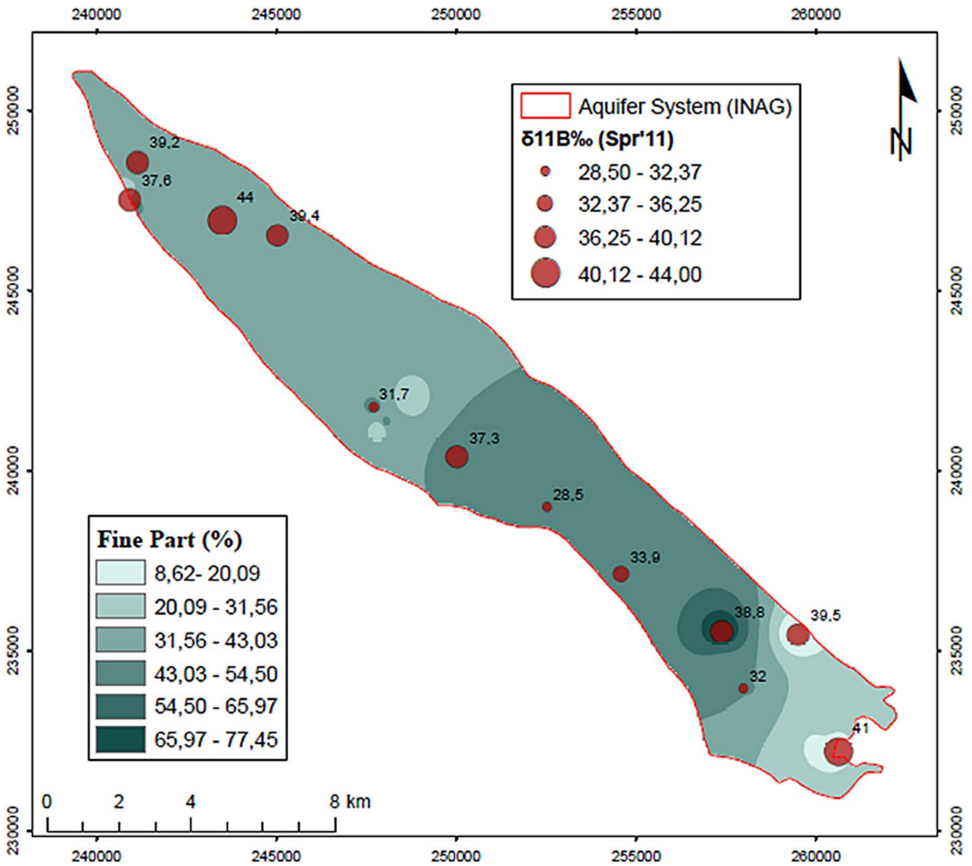


Fig. 11 Distribution of fine content (< 63 µm) in soils of the Monforte-Alter do Chão aquifer system and measured $\delta^{11}B$ (‰) in water samples taken in the spring 2011 sampling campaign

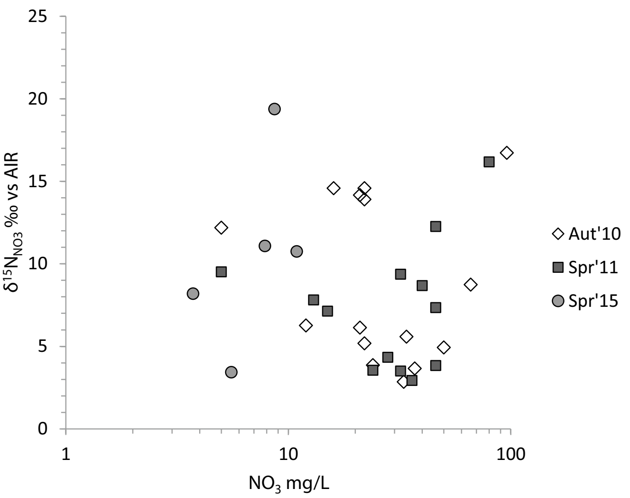


Fig. 12 Isotopic composition of dissolved nitrates, $\delta^{15}N_{NO3}$, vs nitrate concentrations

isotopes as tracers of anthropogenic impact on water have focused on the identification of wastewater and sewage dominated by products synthesized from B (Bassett 1990; Vengosh et al. 1994, 1999; Eisenhut et al. 1996; Sacchi 2013; Basset et al. 1995; Vengosh 1998). Basset et al. (1995) and Komor (1997) were the first to use isotopes as tracers of NO_3^- and B, but only Komor (1997) reports the isotopic signatures of B on inputs related to agriculture (pig manure, livestock effluent from barns, and synthesized fertilizers B: Fig. 16) to distinguish the sources of contamination in NO_3^- in groundwater and surface water.

The $\delta^{11}B$ in the Monforte-Alter do Chão groundwater body ranged from + 28.5 to + 44.0‰ (mean of + 36.9‰) in spring'11. The B concentration in groundwater is lower than 0.046 mg/l, and it is possible to see a slight positive trend between the B concentrations and the $\delta^{11}B$ values. A preliminary analysis of isotopic measurements of $\delta^{11}B$ in selected samples (according to Komor 1997) points to the presence of nitrates derived from effluents of pig farms (Fig. 17). However, considering the land-use and livestock activities in the area of the Monforte-Alter do Chão aquifer system, the presence of contamination originating from pig farms is minor in relation to that from cattle. In the groundwater samples, $\delta^{11}B$ is higher than expected for livestock effluent (30‰ $\delta^{11}B$). It is not possible to observe any correlation between $\delta^{11}B$ and dissolved chloride (Fig. 18a), indicating that high values of $\delta^{11}B$ cannot be explained by simple evaporation processes. According to Widory et al. (2004, 2005) and Tirez et al. (2010), the combined analysis and determination of $\delta^{15}N$ and $\delta^{11}B$ isotope can be used in

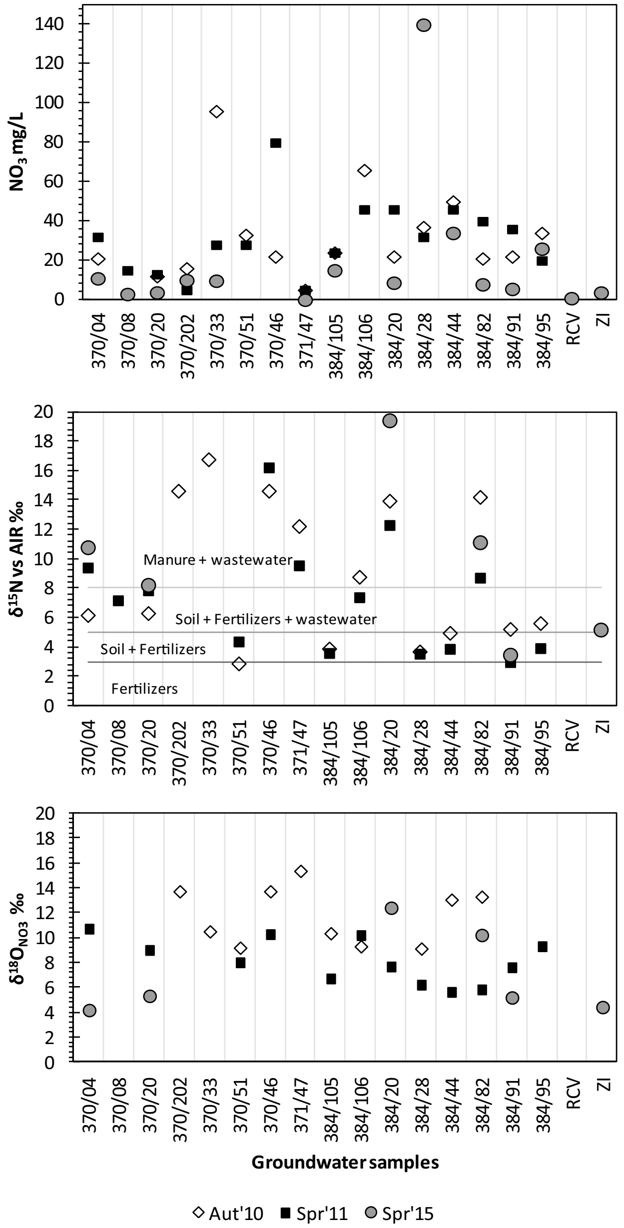


Fig. 13 Variation of δ^{15}N and δ^{18}O in the analyzed nitrates, individualizing by sampling season, and reference values of possible N sources; δ^{15}N soil from Kendall et al. (2007); δ^{15}N animal manure from Vitòria et al. (2004); δ^{15}N wastewater from Aravena et al. (2010); δ^{15}N fertilizers from Michalski et al. (2015)

the identification of origins of nitrate contamination, allowing access to the path and location of the various NO$_3$ inputs in the aquifer system (Fig. 18).

Isotopes of B are not affected by denitrification, maintaining the signature of the sources of solutes, however, can undergo fractionation through processes such as evaporation, crystallization, precipitation, complexation, and adsorption onto clay minerals or ferrihydroxides (Bassett 1990, Bassett et al. 1995). The ^{10}B is preferentially present as B(OH)$_4$, while ^{11}B is enriched in the B(OH)$_3$. The B(OH)$_4$ species are preferably adsorbed by soil and minerals, leading to an enrichment of ^{10}B in the solid phase (fractionated by 30–40‰) when boron is incorporated from aquatic systems by heterogeneous exchange, a concomitant enrichment of ^{11}B occurs in the residual fluids (Tirez et al. 2010). No relation was observed between δ^{11}B and the concentrations of dissolved total iron and manganese, that allowed inferring the occurrence of phenomena of precipitation/adsorption of the isotopes of B by oxides and hydroxides. Although a similar distribution pattern was not observed for the δ^{11}B values and the fine particle content in the soils (Fig. 11), it is possible that the adsorption of B onto clays could theoretically explain the δ^{11}B, taking into account the very low concentrations of B in groundwater. It is also possible to see a positive trend between the δ^{11}B and the pH of the groundwaters (Fig. 18b) and that can be the main reason for the ^{11}B enrichment of the Monforte-Alter do Chão groundwater body. Beyond all this, we must consider that the B isotope composition reflects the animals' diet and physiology varying from region to region (Komor 1997).

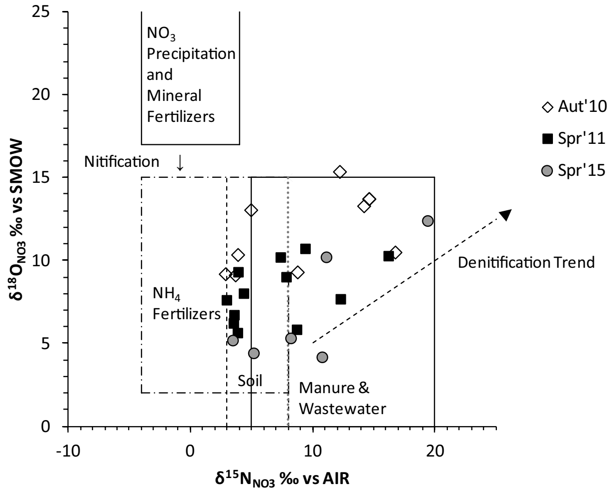


Fig. 14 Stable isotope composition of dissolved nitrates in groundwater; δ^{15}N vs δ^{18}O; fields show typical relations for synthetic or mineral fertilizers, NH$_4$ fertilizers, N from soil, and nitrate from manure and wastewater (Kendall et al. 2007); the trends resulting from nitrate transformation processes' trends, nitrification, and denitrification (Kendall 1998), area represented

6. Conclusions

The groundwaters from Monforte-Alter do Chão aquifer system show widely variable chemical composition, typical of circulation through different lithologies, gabbroic rocks, and carbonates. The majority have short residence time in the aquifer, reflecting physical and chemical alterations

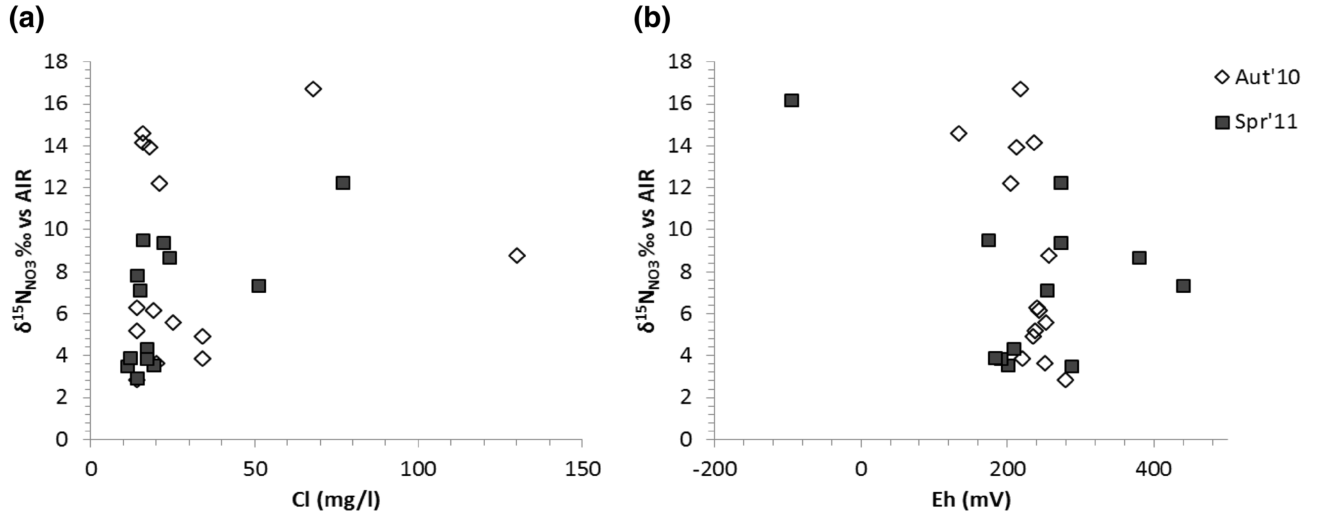


Fig. 15 **a** δ^{15}N vs dissolved chloride in groundwaters; **b** δ^{15}N vs redox potential of the groundwater

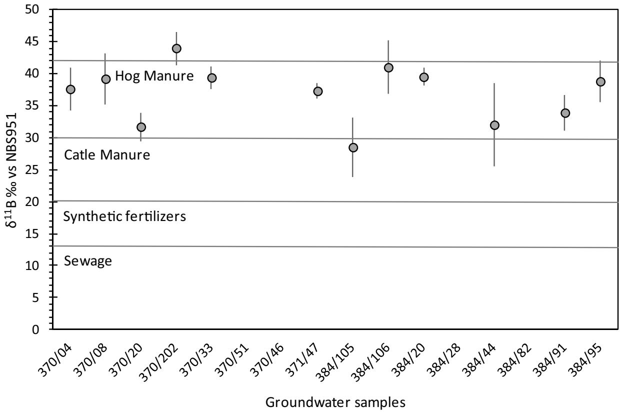


Fig. 16 δ^{11}B values measured on groundwaters sampled in the spring'11 campaign and some reference values (after Komor 1997) used to help partitioning sources of NO_3^- in groundwater

associated with the recharge zone, namely evaporation and nitrogen contamination.

In this study, isotopic composition of NO_3 (δ^{15}N and δ^{18}O) and B (δ^{11}B) was used to identify the sources of the nitrogen contamination.

Despite the occurrence of some evaporation at the soil or vadose zone, the δ^{15}N and δ^{18}O isotopes allowed to distinguish two groups of waters with different sources of nitrates, fertilizers, and soils or manure and domestic effluents. Samples classified as having nitrates from septic tanks or organic fertilizers are associated with urban wastewater, since they are located around major towns. Those whose origin results from application of organic fertilizers are directly dependent on the accumulation of animal waste (livestock production). The samples classified as having N from mixed sources or processes are samples that have high δ^{15}N and δ^{18}O and can

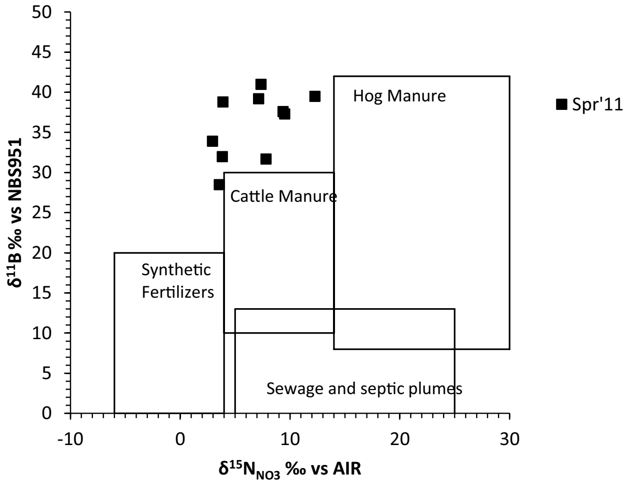


Fig. 17 δ^{11}B vs δ^{15}N of groundwater samples to distinguish the sources of contamination by NO_3 in groundwater; fields after Widory et al. (2004, 2005) and Tirez et al. (2010)

be associated with manure lixiviates or NH_4 fertilizers. As there are no negative correlation between $\delta^{18}O_{NO3}$ and dissolved NO_3^-, the first source will be most likely.

The analysis of δ^{11}B ratio pointed out the presence of nitrogen from effluents of pig farms. However, considering land occupation and livestock activities in the area of the Monforte-Alter do Chão aquifer system, the presence of contamination originated at pig farms has to be minor in relation to what may be expected from cattle. It is possible that the adsorption of ^{11}B isotopes onto clays or the high pH of the groundwater could explain the high δ^{11}B values, and that can be the main reason for the ^{11}B enrichment of the Monforte-Alter do Chão groundwater body, masking the true interpretation of the nitrogen source. Further studies must

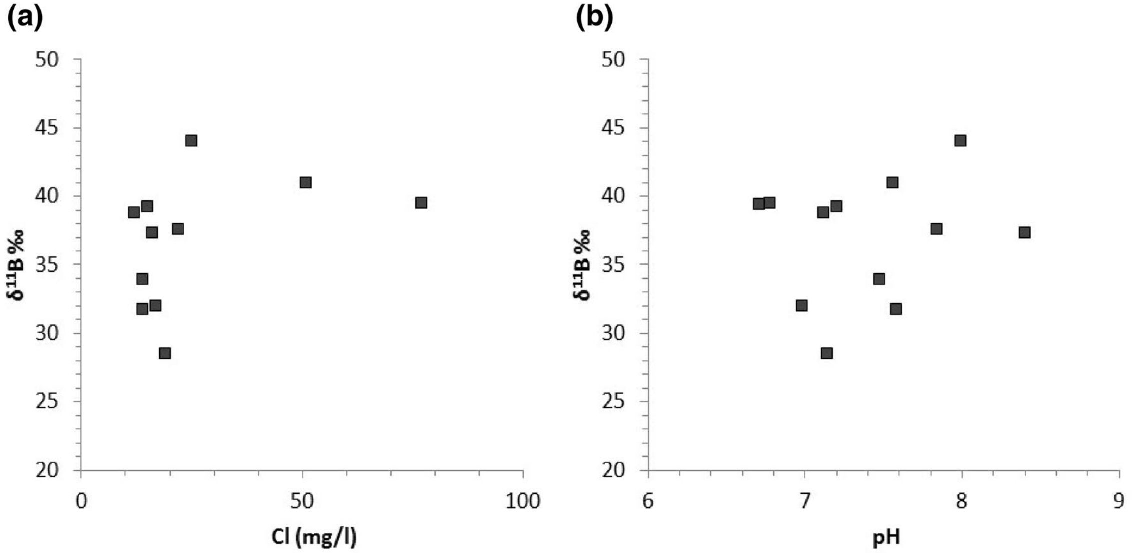


Fig. 18 Groundwater sampled in the spring'11 campaign; **a** δ^{11}B vs dissolved chloride; **b** δ^{11}B vs pH

be carried out investigating chemical composition and the B isotope ratios in soils and not contaminated waters.

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