



UNIVERSITAT POLITÈCNICA
DE CATALUNYA
BARCELONATECH

UPCommons

Portal del coneixement obert de la UPC

<http://upcommons.upc.edu/e-prints>

Aquesta és una còpia de la versió *author's final draft* d'un article publicat a la revista Journal of bone and mineral research.

URL d'aquest document a UPCommons E-prints:

<http://hdl.handle.net/2117/86747>

Article publicat / *Published paper:*

Anna Menció, Josep Mas-Pla, Neus Otero, , Oriol Regàs, Mercè Boy-Roura, Roger Puig, Joan Bach, Cristina Domènech, Manel Zamorano, David Brusi, Albert Folch (2015), Science of the total environment 539: 241–251. doi:10.1016/j.scitotenv.2015.08.151

Elsevier Editorial System(tm) for Science of the Total Environment
Manuscript Draft

Manuscript Number: STOTEN-D-15-01963

Title: Nitrate pollution of groundwater; all right..., but nothing else?

Article Type: Research Paper

Keywords: nitrate pollution, hydrochemistry, nitrate isotopes, water-rock interaction, multivariate analysis, groundwater

Corresponding Author: Dr. Anna Menció, Ph.D.

Corresponding Author's Institution: Universitat de Girona

First Author: Anna Menció, Ph.D.

Order of Authors: Anna Menció, Ph.D.; Josep Mas-Pla, Ph.D; Neus Otero, Ph.D; Oriol Regàs, M.D.; Mercè Boy-Roura, Ph.D; Roger Puig, Ph.D; Joan Bach, Ph.D; Cristina Domenech, Ph.D; Albert Folch, Ph.D

Abstract: Contamination from agricultural sources and, in particular, nitrate pollution, is one of the main concerns in groundwater management. However, this type of pollution entails the entrance of other substances into the aquifer, as well as it may promote other processes. In this study, we deal with hydrochemical and isotopic analysis of groundwater samples from four distinct zones in Catalonia (NE Spain), which include different lithological units, to investigate the influence of manure fertilization on the overall hydrochemical composition of groundwater. Results indicate that high nitrate concentrations, resulting from intense manure application, homogenize the contents of the major dissolved ions (i.e.; Cl⁻, SO₄²⁻, Ca²⁺, Na⁺, K⁺, and Mg²⁺). Moreover, positive linear relationships between nitrate and some ions are found indicating the magnitude of the fertilization impact on groundwater hydrochemistry. Nevertheless, the increasing concentration of specific ions is not only attributed to the manure input, but to the enhancing effect of manure and slurry upon the biogeochemical processes that control water-rock interactions. Such results raise awareness that such processes should be evaluated in advance in order to assess adequate groundwater resources assessment.

Suggested Reviewers: Bernard T Nolan Ph.D

U.S Geological Survey

btnolan@usgs.gov

Dr. B.T. Nolan is an expert on nitrate pollution, and the different methods used to determine nitrate occurrence and distribution (such as logistic regression).

Elisa Sacchi Ph.D

Università degli Studi di Pavia

elisa.sacchi@unipv.it

Dr. E. Sacchi has a wide experience as in environmental geochemistry, and especially, the environmental application of isotopes geochemistry.

Thomas Harter Ph.D

University of California

thharter@ucdavis.edu

Dr. T. Harter is an expert on hydrogeology, especially on groundwater pollution transport.

Corinne Le Gal La Salle
Université de Nîmes
corinne.legallasalle@unimes.fr

Dr. C. Le Gal La Salle has a wide experience in geochemistry and isotopic characterization of natural and polluted waters.

Albert Soler Ph.D
Universitat de Barcelona
albertsolergil@ub.edu

Dr. A. Soler is an expert on nitrate pollution, and especially on the use of nitrate isotopes.

Opposed Reviewers:

Grup de Geologia Aplicada i Ambiental, Àrea de Geodinàmica Externa.

Editor.

Science of the Total Environment

Dear Sir,

We submit the manuscript entitled "Nitrate pollution of groundwater; all right..., but nothing else?", by A. Menció, J. Mas-Pla, N. Otero, O. Regàs, M. Boy-Roura, A. Folch, R. Puig, C. Domenech and J.Bach, to be considered for publication in *Science of the Total Environment*.

Please, address all correspondence to:

Anna Menció, Ph.D.

Grup de Geologia Aplicada i Ambiental (GAiA)
Centre de Recerca en Geologia i Cartografia Ambiental (Geocamb)
Àrea de Geodinàmica Externa,
Departament de Ciències Ambientals,
Universitat de Girona.
Campus de Montilivi
17071 Girona, Spain.

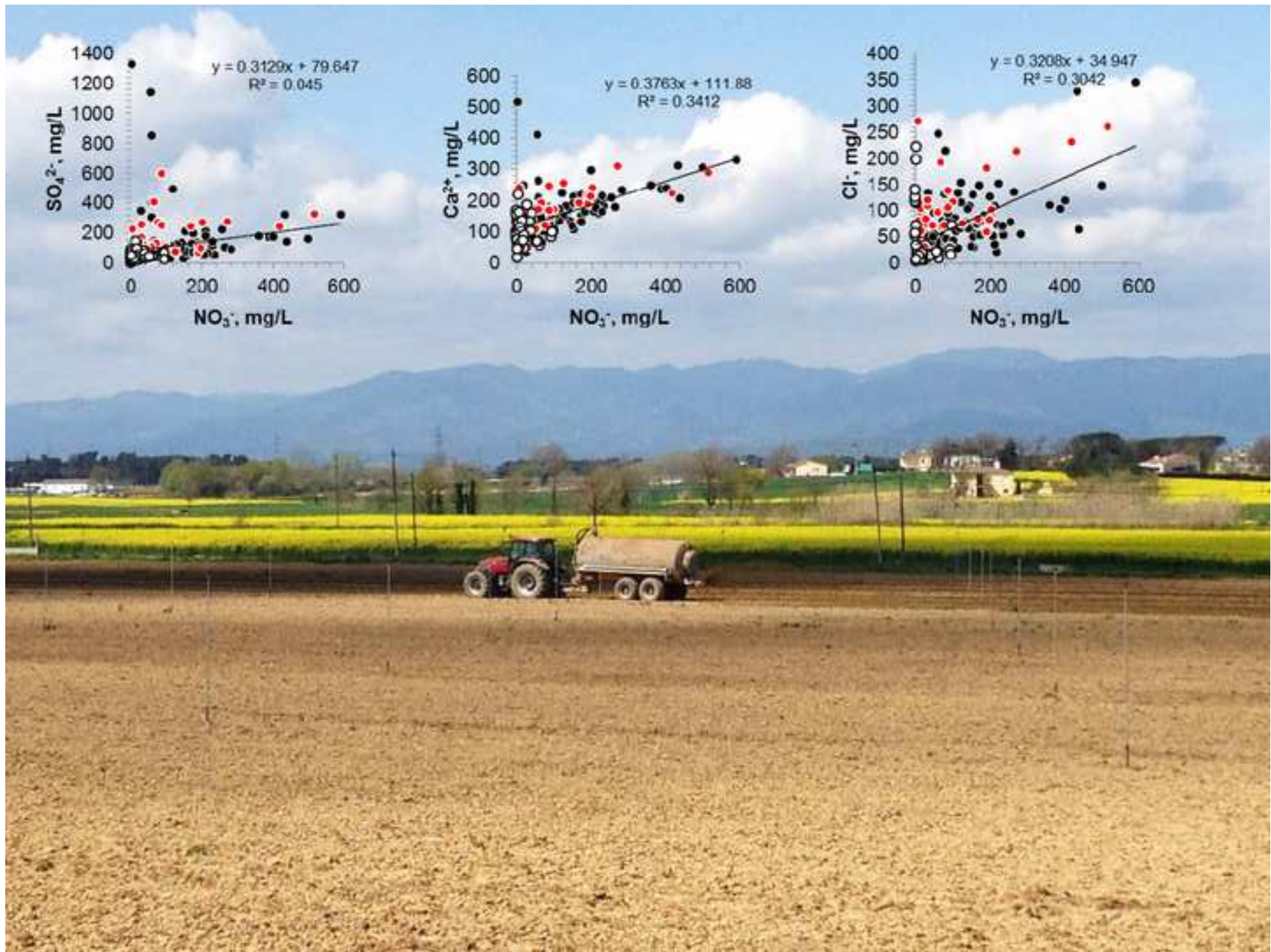
Tel: +34-972-418704
Fax: +34-972-418150
e-mail: anna.mencio@udg.edu

Yours sincerely,



Dr. Anna Menció,

Girona, May 11, 2015.



Highlights

- The effects of nitrate pollution have been evaluated in five different aquifer types
- Statistical and multivariate analyses are used to identify groundwater changes
- Agricultural pollution modifies groundwater conditions and geochemical processes

1 Nitrate pollution of groundwater; all right..., but nothing else?

2
3
4
5
6
7
8 2 *Anna Menció¹, Josep Mas-Pla^{1,2}, Neus Otero³, Oriol Regàs¹, Mercè Boy-Roura², Roger Puig³, Joan*
9
10 3 *Bach⁵, and Cristina Domenech³, Albert Folch⁵*

11
12
13 4 ¹ Grup de Geologia Aplicada i Ambiental (GAiA), Centre de Recerca en Geologia i Cartografia Ambiental (Geocamb), Dept.
14 5 de Ciències Ambientals, Universitat de Girona, Spain, e-mail: anna.mencio@udg.edu

15
16 6 ² Institut Català de Recerca de l'Aigua (ICRA), Spain, email: jmas@icra.cat.

17
18 7 ³ Grup de Mineralogia Aplicada i Geoquímica de Fluids, Dept. de Cristal·lografia, Mineralogia i Dipòsits Minerals, Facultat
19 8 de Geologia, Universitat de Barcelona, Spain, email: notero@ub.edu.

20
21 9 ⁴ Àrea de Geodinàmica Externa i Hidrogeologia, Dept. de Geologia, Universitat Autònoma de Barcelona, Bellaterra, Spain,
22 10 email: joan.bach@uab.cat.

23
24 11 ⁵ Grup d'Hidrologia Subterrània, Dept. D'Enginyeria del Terreny, Cartogràfica i Geofísica. Universitat Politècnica de
25 12 Catalunya-Barcelona Tech, Spain, email: folch.hydro@gmail.com.

26
27 13
28
29 14 *Submitted to: Science of the Total Environment*
30
31

32 15
33
34 16 **KEY WORDS:** nitrate pollution, hydrochemistry, water-rock interaction, multivariate
35
36 17 analysis, groundwater
37
38

39 **ABSTRACT**

40
41
42
43
44 19 Contamination from agricultural sources and, in particular, nitrate pollution, is one of the
45
46 20 main concerns in groundwater management. However, this type of pollution entails the
47
48
49 21 entrance of other substances into the aquifer, as well as it may promote other processes. In
50
51 22 this study, we deal with hydrochemical and isotopic analysis of groundwater samples from
52
53 23 four distinct zones in Catalonia (NE Spain), which include different lithological units, to
54
55
56 24 investigate the influence of manure fertilization on the overall hydrochemical composition of
57
58
59 25 groundwater. Results indicate that high nitrate concentrations, resulting from intense manure
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

application, homogenize the contents of the major dissolved ions (i.e.; Cl^- , SO_4^{2-} , Ca^{2+} , Na^+ , K^+ , and Mg^{2+}). Moreover, positive linear relationships between nitrate and some ions are found indicating the magnitude of the fertilization impact on groundwater hydrochemistry. Nevertheless, the increasing concentration of specific ions is not only attributed to the manure input, but to the enhancing effect of manure and slurry upon the biogeochemical processes that control water-rock interactions. Such results raise awareness that such processes should be evaluated in advance in order to assess adequate groundwater resources assessment.

Highlights

- The effects of nitrate pollution have been evaluated in five different aquifer types
- Statistical and multivariate analyses are used to identify groundwater changes
- Agricultural pollution modifies groundwater conditions and geochemical processes

INTRODUCTION

Nitrate occurrence and transport in aquifers have been widely studied since it is one of the major threats in groundwater, and most aquifers in agricultural areas are affected by this contaminant (Spalding and Exner, 1993; EEA, 2012). Most of the papers that focused on nitrate pollution study, by means of hydrochemical and isotopic data, nitrate trends in groundwater at different scales to identify potential sources of pollution and build-up hydrogeochemical models to understand the behavior of nitrate polluted aquifers (for instance, Burg and Heaton, 1998; Hudak, 2000; Katz et al., 2004; Masetti et al., 2008; Vitòria et al., 2008; Carbó et al., 2009; Kaown, et al., 2009; Menció et al., 2011; Boy-Roura et al., 2013). However, agricultural pollution due to a long and continued application of organic fertilizer (slurry and manure) may not only entail an increase on this ion in aquifers. On the one hand, other substances present in manure also enter into the groundwater system, such as

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

ions, metals, emerging organic contaminants, or even microorganisms. On the other hand, these substances interact with the subsurface environment, modifying groundwater conditions, enhancing geochemical processes and even modifying groundwater communities (Cho et al., 2000; Böhlke, 2002; Murray et al., 2010; Stein et al., 2010; Korbel and Hose, 2011; Lapworth et al., 2012; Korbel et al., 2013; Choi et al., 2013).

In this study, we look for the evidence of the changes that manure application exerts on the major components concentrations by conducting an analysis of a large hydrochemical database, including isotopic data, of nitrate polluted aquifers representative of several geological environments. Our aim is to analyze whether manure and slurry application lead to a loss of the hydrogeological fingerprint of the geological background, and whether the resulting groundwater composition is only caused by mixing with manure lixiviates or by changes in biogeochemical processes.

Manure applied as fertilizer has a complex chemical composition. For instance, Vitòria (2004) analyzed its composition from several farms in Osona (Catalonia, NE Spain) and reported that the concentration of some major inorganic components in pig manure, the most used organic fertilizer in the studied areas in Catalonia (NE Spain), is notably larger than their natural values in groundwater. This is the reason why its impact on groundwater hydrochemistry must be evaluated to avoid misinterpretation of supposedly hydrochemical natural data.

Samples for this study belong to four distinct regions in Catalonia, all of them classified as Nitrate Vulnerable Zones (NVZs) as a result of the transposition of the Nitrate Directive (ND) 91/676/EC. In fact, NVZs in Catalonia cover up to 40% of the total area, and half of them show nitrate concentrations higher than 40 mg/L, affecting 17 out of the 53 groundwater bodies which are at risk of not meeting the European Water Framework Directive goals (ACA, 2007; Boy-Roura, 2013). In this study, we analyze datasets from the

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

75 following NVZs, according to published results in the Selva basin (Folch et al., 2011; Menció
76 et al., 2012; Puig et al., 2013), Empordà basin (Puig, 2014), Osona region (Vitòria et al.,
77 2008; Otero et al., 2009; Menció et al., 2011; Boy-Roura et al., 2013), and Garrotxa area
78 (based on still unpublished data). According to their lithologies, five groups of aquifers are
79 distinguished (Figure 1 and Figure 2):

- 80 • Group 1: Aquifers in igneous rocks, especially granite and granodiorite and, in a
81 lesser degree, in metamorphic rocks such as shale, schist, marble, and gneiss. These
82 aquifers are mainly located in the ranges surrounding the Selva and Empordà basins.
83 Hydrochemically, they present $\text{Ca}^{2+}\text{-HCO}_3^-$ and $\text{Ca}^{2+}\text{-HCO}_3^-\text{-Cl}^-$ facies, tending to
84 evolve to $\text{Na}^+\text{-HCO}_3^-$.
- 85 • Group 2: Aquifers in sedimentary rocks, mainly Paleogene sedimentary rocks,
86 including detritic, organic and chemical sedimentary rocks, such as conglomerates,
87 sandstones, siltstones, limestones, marls, and gypsum deposits. Although these
88 aquifers are present in all the study areas, they are mainly exploited in Osona and
89 Garrotxa, and with a minor extent in Empordà. Main hydrochemical facies are $\text{Ca}^{2+}\text{-}$
90 HCO_3^- , $\text{Ca}^{2+}\text{-Mg}^{2+}\text{-HCO}_3^-$ or $\text{Ca}^{2+}\text{-SO}_4^{2-}$.
- 91 • Group 3: Aquifers in sediments derived from igneous and metamorphic rocks. The
92 arkosic sands, gravels and silt layers of the Neogene deposit that filled the Empordà
93 and Selva areas, and the more recent Quaternary alluvial formations associated to the
94 main rivers in these basins, are the weathering products of the main range areas,
95 mainly located in the Pyrenees Montseny-Guilleries and Gavarres ranges. They show
96 similar facies than Group 1.
- 97 • Group 4: Aquifers in sediments derived from sedimentary rocks. These aquifers are
98 located in Osona and Garrotxa basins, and constitute the Quaternary alluvial aquifers
99 and surface formations in these areas. Their facies are analogous to those of Group 2.

- Group 5: Aquifers in volcanic materials. In this group of aquifers there is a broad variety of materials, from phreatomagmatic and pyroclastic deposits to basaltic lava flow locally interleaved by sedimentary (alluvial) levels, which may locally be the most productive units. These materials are mainly located in the Garrotxa area, but volcanic lithologies also constitute aquifers in some parts of the Selva basin. This group shows the lowest EC values with Ca^{2+} - HCO_3^- or Ca^{2+} - Mg^{2+} - HCO_3^- facies.

METHODOLOGY

Field surveys, in the studied datasets (Folch et al., 2011; Menció et al., 2012; Puig et al., 2013; Puig, 2014; Otero et al., 2009; Menció et al., 2011; Boy-Roura et al., 2013), were conducted from 2006 to 2013. A total number of 204 groundwater sampling locations constitute the whole dataset –distributed as Selva basin: 37 samples; Empordà basin: 45; Osona region: 57; Garrotxa area: 65– and they are classified according to the five described lithological groups (Table 1).

Similar analytical procedures for hydrochemical and isotopic analysis were followed at each studied region. Specific details are described in each of the given references. Concentration units are reported in mg/L. Most of the samples had an ionic mass balance error between $\pm 5\%$. Isotope δ -notation for water isotopes is expressed in terms of the ‰ deviation of the isotope ratio of the sample relative to that of the V-SMOW standard. Analytical errors are $\pm 0.06\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 0.7\text{‰}$ for δD . Nitrate isotope notation is also expressed in terms of δ (‰) relative to that of the international standards AIR (atmospheric N_2) for $\delta^{15}\text{N}$. Precision ($\equiv 1\sigma$) of the samples are $\pm 0.3\text{‰}$ and $\pm 0.4\text{‰}$, for $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$, respectively.

The SPSS program (version 19, 2010, SPSS Inc.) was used to conduct the different statistical analyses. Differences between aquifer hydrochemical parameters were analyzed considering the Kruskal-Wallis and Mann-Whitney U tests, for non-parametric data, since none of the

125 parameters were normally distributed. In addition, a Principal Component Analysis (PCA)
126 was conducted in order to identify associations between variables and samples. The
127 associations obtained through a PCA, based on similar magnitudes and variations in
128 chemical, physical, and isotopic values in the groundwater samples composition, are adequate
129 to indicate the influence of human factors, hydrochemical processes, or even, the origin of
130 groundwater (for instance, Helena et al., 2000; Menció and Mas-Pla, 2008; Menció et al.,
131 2013; Re et al., 2014).

133 **RESULTS AND DISCUSSION**

134 At a first glance, hydrochemical characteristics of groundwater samples with low NO_3^-
135 content (below the health standard limit of 50 mg NO_3^-/L) reflect the dominant role of
136 lithology in the composition, as observed in Figure 2. Mean and standard deviation values
137 (Table 1) and boxplot shapes of selected parameters (EC , Cl^- , SO_4^{2-} , Na^+ , Ca^{2+} ; Figure 2) for
138 samples with low nitrate content, reflect the differences among aquifer types attributed to
139 distinct lithology, showing the hydrochemical characteristics of groundwater samples under
140 natural conditions. For instance, aquifers in groups 1 and 3, including igneous and
141 metamorphic aquifers and sediments derived from these rocks, do not show significant
142 differences between concentrations of the major hydrochemical components (with p-values
143 between 0.089 and 0.881). These aquifers, for instance, present significant differences when
144 compared with groups 2 and 4, representing aquifers in sedimentary rocks and in sediments
145 derived from these rocks, for ions such as Cl^- , Na^+ , and Ca^{2+} (p-values ranging from values
146 <0.001 to 0.038). Contrarily, hydrochemical characteristics of groundwater at high-nitrate
147 concentrations (> 50 mg NO_3^-/L ; Figure 2) seem to homogenize their concentrations despite
148 lithological differences, presenting wider ranges for major ions distribution and erasing the

149 main differences in their hydrochemical composition of the distinct groups observed at low
150 NO_3^- samples.

151 In detail, when samples of low and high nitrate content for each lithological aquifer group are
152 compared, significant higher EC values are observed in the most polluted wells (p-values
153 ranging from 0.006 to values lower than 0.001). Higher EC values are related to significant
154 higher concentrations of ions: Cl^- , SO_4^{2-} , Na^+ and Ca^{2+} (Table 1). An exception are samples
155 of group 1, which do not show significant differences in any of these parameters between low
156 and high nitrate groups (p-values ranging from 0.123 for Ca^{2+} to 0.877 for Cl^-), since some of
157 these samples belong to thermal and/or CO_2 -rich systems. Geochemical evolution in such
158 environments overlays the chemical contribution of manure inputs. In addition, it is worth
159 mentioning that these Cl^- and Na^+ higher concentrations are not detected in wells located in
160 aquifer group 3 (in sediments derived from igneous and metamorphic rocks, and p-values of
161 0.684 and 0.844, respectively), where natural processes can also favor high Cl^- and Na^+
162 concentrations.

163 It is also relevant that fewer hydrochemical differences are detected when high nitrate
164 concentration sample populations for the different aquifers are compared (Figure 2 and Table
165 1). As expected due to lithological similarity, no significant differences are detected between
166 groups 1 and 3 (p-values ranging from 0.087 for SO_4^{2-} and 0.906 for Cl^-), neither between
167 groups 2 and 4 (p-values between 0.050 for HCO_3^- and 0.732 for SO_4^{2-}). Nevertheless, when
168 samples with high nitrate concentrations of group 1 and 2 are compared, only significant
169 differences in SO_4^{2-} and K^+ are detected (p-values of 0.024 and 0.023), while other
170 components show similar concentration ranges. Furthermore, differences among high nitrate
171 concentrations samples of groups 1 and 4 are only evident for Ca^{2+} and K^+ (p-values of 0.018
172 and 0.011); comparing samples of groups 2 and 3, significant differences are reduced to Mg^{2+}
173 and K^+ (with p-values <0.001); and, differences between samples of groups 1 and 5 with high

174 nitrate concentrations are significant for Na^+ and K^+ (p-values of 0.014 in both cases). Indeed,
175 this group 5 shows the largest differences with the rest of the aquifer groups for high NO_3^-
176 samples. In volcanic materials, nitrate pollution is clearly lower than in the rest of the studied
177 aquifers, with an overall mean value of 30.90 ± 3.81 mg/L (Table 1). In this case, high nitrate
178 concentration samples presented differences with respect to nitrate for samples of groups 2, 3
179 and 4 (p-values of 0.026, 0.027 and 0.031, respectively). Between group 5 and these other
180 groups, significant differences were also observed in EC, Cl^- , Ca^{2+} and SO_4^{2-} (p-values
181 ranging from <0.001 to 0.044); besides, significant differences were detected between group
182 5 and groups 2 and 3 for Ca^{2+} (p-values <0.001); and, with group 3 for K^+ and Mg^{2+} (p-values
183 of 0.008 and 0.004, respectively) .

184 Complementarily, two Principal Component Analyses were conducted to determine the
185 different relationships among samples and variables. The first one considered all available
186 samples, a total number of 204; and considered eleven variables, including: EC, pH, total
187 aqueous concentration of HCO_3^- , Cl^- , SO_4^{2-} , Ca^{2+} , Mg^{2+} , Na^+ and K^+ , and $\delta^{18}\text{O}$ and δD . In the
188 second analysis, the dataset included only those samples that, in addition to the mentioned
189 variables, also included nitrate isotopic data ($\delta^{15}\text{N}_{\text{NO}_3}$). In this analysis the number of samples
190 was reduced to 158, as in 32% of them (mainly from group 5) lacked the nitrate isotopic
191 information. By conducting both statistical analyses we seek illustrating whether
192 denitrification processes are relevant on the understanding of the hydrochemical changes in
193 groundwater related to nitrate pollution. This is the reason why both analyses are shown
194 herein, and compared.

195 In the first PCA, variables as pH, HCO_3^- , K^+ and δD were ruled out of in order to obtain
196 better values of the goodness-of-fit statistics. Thus, a PCA conducted with the 7 remaining
197 variables had a Barlett chi-square statistic of 1241.6 (for 28 degrees of freedom and a
198 minimum significance level of <0.001), and a value of sampling adequacy (MSA) obtained

199 by the Kaiser-Meyer-Olkin of 0.732. In the second analysis, the final solution considered 8
200 variables, and had a better fit with a Barlett chi-square statistic of 1085.5 (for 36 degrees of
201 freedom and a minimum significance lower than 0.001), and a MSA of 0.758.

202 Four varifactors (VF) were obtained in both analyses, explaining 91.59% and 88.19% of the
203 total variance, respectively. In each PCA, these varifactors explained the same processes and
204 associations of variables; excepting natural attenuation, which was only considered in the
205 second one as $\delta^{15}\text{N}_{\text{NO}_3}$. For this reason, only the scores and samples distribution obtained in
206 the second PCA are shown in Table 2 and Figure 3. Thus, after a Varimax rotation, the final
207 VFs were interpreted as follows:

- 208 • VF1 includes SO_4^{2-} , Mg^{2+} , Ca^{2+} , and EC, explaining a 32.53% of the total variance.
209 This VF1 stands for water-rock interaction processes, specifically in sedimentary
210 formations where gypsum dissolution occurs, since VF1 highest values are obtained
211 in samples located in aquifers belonging to groups 2 and 4, where these processes
212 have been described to govern groundwater hydrochemistry (Figure 3a; Menció et al.,
213 2011; Soler et al., 2014).
- 214 • VF2 represents the 20.41% of variability of the data. It mainly includes NO_3^- , with a
215 lower participation of Ca^{2+} , EC and Cl^- . VF2 has been interpreted as nitrate pollution,
216 showing the direct relationship between NO_3^- and EC, Ca^{2+} and Cl^- variables.
- 217 • With a 20.23% of the total variance, VF3 is participated by $\delta^{18}\text{O}$, Na^+ and Cl^- . This
218 VF3 is linked to the recharge altitude of groundwater samples, with high scores in
219 samples recharged at low altitude, and low scores in samples recharged at high
220 altitude. In addition, it is worth recalling that recharge areas of most of the wells
221 drilled in igneous and metamorphic rocks and in the sediments derived from them
222 (groups 1 and 3) are located at low altitude. Because of their lithology, water samples

223 present high concentrations of Na^+ and Cl^- , explaining the association of both ions
224 with $\delta^{18}\text{O}$ (Table 1, Figure 2 and Figure 3).

- 225 • VF4 is mainly associated to $\delta^{15}\text{N}_{\text{NO}_3}$, with a lower participation of Na^+ and Cl^- . With a
226 15.03% of the total variance explained, this last VF represents natural attenuation
227 processes (i.e., denitrification). The association of Na^+ and Cl^- with high $\delta^{15}\text{N}_{\text{NO}_3}$
228 values ($\delta^{15}\text{N} > 15\text{‰}$, according to Kendall et al., 2007) may be attributed to the
229 attenuation processes described in the Selva basin, group 3, where regional flow
230 systems with $\text{Na}^+\text{-HCO}_3^-$ facies and reducing conditions, enhanced natural
231 heterotrophic attenuation processes (Puig et al., 2013).

232 Two sample tendencies with positive scores for SO_4^{2-} , Ca^{2+} and Mg^{2+} (that is, VF1) are
233 distinguished in a plot VF1 vs. VF2. The first tendency presents high scores for VF2 as well,
234 which links samples with high SO_4^{2-} , Ca^{2+} and Mg^{2+} content to high nitrate concentrations
235 (quadrant I in Figure 3a), including samples from aquifer groups 2, 3 and 4 with nitrate
236 concentrations between 280 to 590 mg NO_3^-/L . The second one is associated to negative
237 values in VF2, and it is composed of samples from aquifer groups 2 and 4, those related to
238 sedimentary rocks and the sediments derived from these materials. In this case, nitrate
239 concentrations are notably lower, between 2.5 to 119 mg/L, and the sample with the highest
240 VF1 score is the one with the lowest nitrate concentration. Thus, two clear sets of samples
241 with distinct origins for SO_4^{2-} are distinguished in these study areas: one with SO_4^{2-} related to
242 nitrate pollution, and the other with SO_4^{2-} related to natural sources.

243 In Figure 3b, high and low scores for nitrate pollution (VF2) are distributed in both high and
244 low altitude recharge areas (as defined by $\delta^{18}\text{O}$ isotopic compositions, VF3). This indicates a
245 widespread nitrate distribution all over the study areas, being consistent with an intense
246 agricultural activity. However, most of samples in aquifer group 5, which wells are located in

247 volcanic rocks, present low VF2 values, consistent with the comparatively low pollution
1
2 248 levels in this aquifer type (Table 1).
3

4
5 249 Finally, when nitrate content (VF2) and nitrate attenuation (VF4) are plotted (Figure 3c),
6
7 250 samples with the highest nitrate concentration (with values exceeding 150 mg NO₃⁻/L at VF2
8
9 251 scores > 1) are located in quadrants I and II; while quadrants I and IV include those samples
10
11 252 affected by natural attenuation (with δ¹⁵N>15‰). Samples of quadrant I and IV indicate that
12
13 253 attenuation processes are active independently of the nitrate concentration. Besides, in some
14
15 254 cases, nitrate content has been reduced to values lower than the health standard limits of 50
16
17 255 mg/L, as represented by the sample with negative scores of VF2. These nitrate attenuation
18
19 256 processes have been detected in all aquifers types.
20
21
22
23

24 257 Bivariate plots of selected ions against nitrate concentrations point out their linear increase
25
26 258 proportional to manure application. SO₄²⁻ and Ca²⁺ (both included in VF1) and Cl⁻
27
28 259 (participating in VF2 and VF3) as major components illustrate this fact (Figure 4). Linear
29
30 260 regression equations are estimated for each aquifer lithology using all the available data,
31
32 261 despite their δ¹⁵N_{NO₃} value. Such regression equations prevail over two facts: 1) the inherent
33
34 262 variability of the manure chemical composition depending on its origin and storage before
35
36 263 application; and 2) the heterogeneity of soil processes, which are different, in type and
37
38 264 magnitude, depending on the soil nature and the crop type. Therefore, equations indicate the
39
40 265 rate of ion concentration changes with increasing nitrate content (slope); that is, the amount
41
42 266 of each ion that any unit of nitrate adds to groundwater whether as a direct input or as a result
43
44 267 of enhancing geochemical processes; and the expected value of the major component where
45
46 268 no fertilization occurs (y-intercept). Such linear increase is quite evident for these three ions,
47
48 269 and similar relationships occur for other major components.
49
50
51
52
53
54
55

56 270 Nevertheless, each geological environment show distinct responses to nitrate inputs. Water-
57
58 271 rock interaction in igneous and metamorphic rocks (group 1) will depend on the low
59
60
61
62
63
64
65

272 solubility of silicate minerals, and of other processes as cation exchange, which may
273 significantly alter the $\text{Ca}^{2+}:\text{Na}^+$ ratio and it may be responsible for a wide dispersion of data
274 values with and without the occurrence of nitrate pollution. The large heterogeneity of
275 volcanic deposits (group 5), which may include sedimentary layers among volcanic episodes,
276 also originates a wide range of geochemical values as regards of SO_4^{2-} and Ca^{2+} . However,
277 Cl^- concentration in the studied aquifers appears to be well related to manure fertilization.
278 Moreover, the occurrence of thermal and/or CO_2 -rich waters in group 1 aquifers may enhance
279 specific weathering processes resulting, for instance, in large chloride concentrations that
280 infiltrating water may dilute.

281 As already suggested by the PCA results, SO_4^{2-} content may originate from evaporitic rocks
282 (gypsum) dissolution or from manure contribution. These two geochemical paths are also
283 recognized in groups 2 and 4, where sedimentary rocks or their denudation deposits constitute
284 the aquifers: significantly large values of SO_4^{2-} are found at low nitrate concentration (<75
285 $\text{mg NO}_3^-/\text{L}$), whereas a clear linear relationship between them appears as nitrate content
286 increases. Correlation factors, as they appear in Figure 4, are misleading since they represent
287 the whole dataset; exclusion of those points largely affected by gypsum dissolution will turn
288 to larger R^2 values proving the influence of manure fertilization on the final groundwater
289 composition.

290 Denitrification processes also affect such bivariate relationships. In those places where
291 autotrophic nitrate reduction has been proved (group 2 samples, Osona region; Otero et al.,
292 2009), an increase of SO_4^{2-} due to pyrite oxidation will occur coupled to nitrate decrease;
293 whereas heterotrophic attenuation processes will increase alkalinity. Both processes will also
294 have consequences on the gypsum and calcite equilibriums modifying the Ca^{2+} concentration,
295 yet the neat linear increase of Ca^{2+} with nitrate in most of the aquifer types might suggest that
296 it directly originates in the manure itself. However, a geochemical insight to calcium

297 equilibrium indicates that nitrification of reduced N from manure generates acidity along with
298 NO_3^- and it enhances carbonate dissolution. This process can even be accentuated where lime
299 or dolomite are applied as soil neutralizers in agricultural lands (Böhlke, 2002; Choi et al.,
300 2013), increasing Ca^{2+} and Mg^{2+} concentrations. Complementarily, the analysis of
301 hydrochemical evolution of pig manure stored in experimental pits showed a decrease of Ca^{2+}
302 and Mg^{2+} through time (Vitòria, 2004). Since manure is usually stored for several months
303 before its application, Ca^{2+} increase shown in Figure 4 may be better attributed to
304 geochemical reactions occurring in the soil and in the subsurface following fertilization than
305 to direct manure contribution. Hence, the observed hydrochemical composition is not just a
306 simple conservative mixture of groundwater with infiltrating slurry and manure lixiviation.
307 Contrarily, Na^+ and K^+ showed a conservative behavior in the liquid manure, being their
308 concentrations increased by evaporation (Vitòria, 2004). However, groundwater samples are
309 not specifically rich in both cations (Figure 2), suggesting that other processes as plant
310 uptake, sorption and cation exchange may control their final content in groundwater (Böhlke,
311 2002).

313 CONCLUSIONS

314 Data from aquifers with distinct lithological environments prove that manure application as
315 fertilizer modifies groundwater hydrochemical composition, turning it notably distinct than
316 the expected natural background given by water-rock interaction and other natural processes.
317 Manure and slurry fertilization homogenize the overall hydrochemistry despite lithological
318 differences, hindering the geochemical interpretation inherent to any regional groundwater
319 resources evaluation study.
320 Nevertheless, such compositional modifications which, in general, tend to adopt a linear
321 increase (Figure 4, all data) cannot be solely attributed to the effect of direct manure inputs,

322 as fertilization may enhance or reduce geochemical processes that control groundwater
323 composition at equilibrium. Therefore accepting that nitrate pollution influences,
324 groundwater regional reports should look for the relationships shown in these aquifers;
325 checking potential influences upon the expected (natural) hydrochemical composition, so a
326 better understanding of the hydrogeological system is attained.

328 **ACKNOWLEDGMENTS**

329 This study has been funded by the Spanish Government CGL2011-29975-c04/01 and /04
330 projects and the 2014SGR-1456 project from the Catalan Government. We want to thanks
331 the collaboration of Natural Park of Volcanic Zone Garrotxa, and the Regional Council of
332 Osona.

334 **REFERENCES**

- 335 ACA (Agència Catalana de l'Aigua). Evolució i estat de les aigües subterrànies de Catalunya
336 a conseqüència de la contaminació per nitrats. Generalitat de Catalunya, Departament de
337 Medi Ambient i Habitatge. Barcelona. 2007.
- 338 Böhlke, J.-K. Groundwater recharge and agricultural contamination. *Hydrogeology Journal*,
339 2002, 10:153-179. DOI:10.1007/s10040-011-0183-3
- 340 Boy-Roura, M. Nitrate groundwater pollution and aquifer vulnerability: the case of the Osona
341 region. PhD dissertation. Universitat de Barcelona, 2013.
- 342 Boy-Roura, M.; Menció, A.; Mas-Pla J. Temporal analysis of spring water data to assess
343 nitrate inputs to groundwater in an agricultural area (Osona, NE Spain). *Science of the Total
344 Environment*, 2013, 452-453:433-445. DOI: 10.1016/j.scitotenv.2013.02.065

1 345 Boy-Roura, M.; Nolan, B.T.; Menció, A.; Mas-Pla, J. Regression model for aquifer
2 346 vulnerability assessment of nitrate pollution in the Osona región (NE Spain). Journal of
3
4 347 Hydrology, 2013, 505:150-162.
5
6
7
8 348 Burg, A.; Heaton, T.H.E. The relationship between the nitrate concentration and hydrology of
9
10 349 a small chalk spring; Israel. Journal of Hydrology, 1998, 204: 68–82.
11
12
13
14 350 Carbó, L.I.; Flores, M.C.; Herrero M.A. Well site conditions associated with nitrate
15
16 351 contamination in a multilayer semiconfined aquifer of Buenos Aires, Argentina.
17
18 352 Environmental Geology, 2009, 57:1489–1500.
19
20
21
22 353 Cho, J.; Kim, S. Increases in bacteria community diversity in subsurface aquifers receiving
23
24 354 livestock wastewater input. Applied Environmental Microbiology, 2000, 66:956-965.
25
26 355 DOI:10.1128/AEM.66.3.956.965.2000
27
28
29
30 356 Choi, B.-Y.; Yun, S.-T.; Kim, K.-H.; Choh, S.-J. Geological controlled agricultural
31
32 357 contamination and water-rock interaction in an alluvial aquifer: results from hydrochemical
33
34 358 study. Environmental Earth Sciences, 2013, 68:203-217. DOI:10.1007/s12665-012-1731-y
35
36
37
38 359 EEA (European Environmental Agency) European waters-assessment status and pressures.
39
40 360 EEA Report, No 8/2012. Copenhagen, Denmark, 2012.
41
42
43
44 361 Folch, A.; Menció, A.; Puig, R.; Soler, A.; Mas-Pla, J. Groundwater development effects on
45
46 362 different scale hydrogeological systems using head, hydrochemical and isotopic data and
47
48 363 implications for water resources management: The Selva basin (NE Spain). Journal of
49
50 364 Hydrology, 2011, 403(1):83-102.
51
52
53
54 365 Helena, B.; Pardo, R.; Vega, M.; Barrado, E.; Fernandez, J.M.; Fernandez, L. Temporal
55
56 366 evolution of ground water composition in an alluvial aquifer (Pisuerga River, Spain) by
57
58 367 principal component analysis. Water Resources, 2000, 34, 807–816.
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

368 Hudak, P.F. Regional trends in nitrate content of Texas groundwater. *Journal of Hydrology*,
369 2000, 228:37–47.

370 Kaown, D.; Koh, D.-C.; Lee, K.-K. Effects of groundwater residence time and recharge rate
371 on nitrate contamination deduced from $\delta^{18}\text{O}$, δD , $3\text{H}/3\text{He}$ and CFCs in a small agricultural
372 area in Chuncheon, Korea. *Journal of Hydrology*, 2009, 366:101–111.

373 Katz, B.G.; Chelette, A.R.; Pratt, T.R. Use of chemical and isotopic tracers to assess nitrate
374 contamination and ground-water age, Woodville Karst Plain, USA. *Journal of Hydrology*,
375 2004, 289:36–61.

376 Kendall, C.; Elliott, E.M.; Wankel, S.D. Tracing anthropogenic inputs of nitrogen to
377 ecosystems, Chapter 12, In: R.H. Michener and K. Lajtha (Eds.), *Stable Isotopes in Ecology*
378 *and Environmental Science*, 2007, 2nd edition, Blackwell Publishing, p. 375-449.

379 Korbel, K.L.; Hancock, P.J.; Serov, P.; Lim, R.O.; Hose, G.C. Groundwater ecosystems vary
380 with land use across a mixed agricultural landscape. *Journal of Environmental Quality*, 2013,
381 42:380-390.

382 Korbel, K.L.; Hose, G.C. A tiered framework for assessing groundwater ecosystems health.
383 *Hydrobiologia*, 2011, 661:329-349. DOI:10.1007/s10750-010-0541z

384 Lapworth, D.J.; Baran, N.; Stuart, M.E.; Ward, R.S. Emerging organic contaminants in
385 groundwater: A review of sources, fate and occurrence. *Environmental Pollution*, 2012,
386 163:287–303.

387 Masetti, M.; Poli, S.; Sterlacchini, S.; Beretta, G.P.; Facchi, A. Spatial and statistical
388 assessment of factors influencing nitrate contamination in groundwater. *Journal of*
389 *Environmental Management*, 2008, 86:272–281.

390 Menció, A.; Boy, M.; Mas-Pla, J. Analysis of vulnerability factors that control nitrate
1
2 391 occurrence in natural springs (Osona Region, NE Spain). *Science of the Total Environment*,
3
4 392 2011, 409:3049-3058. DOI: 10.1016/j.scitotenv.2011.04.048
5
6
7
8 393 Menció, A.; Folch, A.; Mas-Pla, J. Identifying key parameters to differentiate groundwater
9
10 394 flow systems using multifactorial analysis. *Journal of Hydrology*, 2012, 472-473:301-313.
11
12 395 DOI: 10.1016/j.jhydrol.2012.09.030
13
14
15
16 396 Menció, A.; Korbel, K.L.; Hose, G.C. River-aquifer interactions and their relationship to
17
18 397 stygofauna assemblages: A case study of the Gwydir River alluvial aquifer (New South
19
20 398 Wales, Australia). *Science of the Total Environment*, 2014, 479-480:292-305. DOI:
21
22 399 10.1016/j.scitotenv.2014.02.009
23
24
25
26 400 Menció, A.; Mas-Pla, J. Assessment by multivariate analysis of surface water -groundwater
27
28 401 interactions in urbanized Mediterranean streams. *Journal of Hydrology*, 2008, 352 (3): 355-
29
30 402 366. DOI:10.1016/j.jhydrol.2008.01.014
31
32
33
34
35 403 Menció, A.; Mas-Pla, J.; Otero, N.; Soler, A. Nitrate as a tracer of groundwater flow in a
36
37 404 fractured multi-layered aquifer. *Hydrological Sciences Journal*, 2011, 56(1):108-122.
38
39 405 DOI:10.1080/02626667.2010.543086
40
41
42
43 406 Murray, K.E.; Thomas, S.M.; Bodour, A. Prioritizing research for trace pollutants and
44
45 407 emerging contaminants in the freshwater environment. *Environmental Pollution*, 2010,
46
47 408 158:3462–71.
48
49
50
51 409 Otero, N.; Torrentó, C.; Soler, A.; Menció, A.; Mas-Pla, J. Monitoring groundwater nitrate
52
53 410 attenuation in a regional system coupling hydrogeology with multi-isotopic methods: the case
54
55 411 of Plana de Vic (Osona, Spain). *Agriculture Ecosystems and Environment*, 2009, 133:103–
56
57 412 113. DOI: 10.1016/j.agee.2009.05.007
58
59
60
61
62
63
64
65

1
2
3
4
5 413 Puig, R. (2014). Multi-isotopic and statistical approaches to trace nitrate pollution sources
6
7
8 414 and assess natural attenuation in groundwater: examples from nitrate vulnerable zones in
9
10 415 Catalonia (NE Spain). PhD Dissertation, Universitat de Barcelona.
11
12
13 416 Puig, R.; Folch, A.; Menció, A.; Soler, A.; Mas-Pla, J. Multi-isotopic study (15N, 34S, 18O,
14
15 417 13C) to identify processes affecting nitrate and sulfate in response to local and regional
16
17 418 groundwater mixing in a large-scale flow system. *Applied Geochemistry*, 2013, 32:129-141.
18
19 419 DOI: 10.1016/j.apgeochem.2012.10.014
20
21 420 Re, V.; Sacchi, E.; Mas-Pla, J.; Menció, A.; El Amrani, N. Identifying the effects of human
22
23 421 pressure on groundwater quality to support water management strategies in coastal regions: A
24
25 422 multi-tracer and statistical approach (Bou-Areg region, Morocco). *Science of the Total*
26
27 423 *Environment*, 2014, 500–501: 211–223. DOI: 10.1016/j.scitotenv.2014.08.115
28
29 424 Soler, D.; Zamorano, M.; Roqué, C.; Menció, A.; Boy, M.; Bach, J.; Brusi, D.; Mas-Pla J.
30
31 425 Evaluación de la influencia de las estructuras tectónicas en la recarga del sistema
32
33 426 hidrogeológico de la depresión del Alt Empordà (NE España) en base a datos hidroquímicos
34
35 427 e isotópicos. II Congreso Ibérico de las Aguas Subterráneas, CIAS2014, 2014.
36
37
38
39 428 Spalding, R.F; Exner, M. E. Occurrence of nitrate in ground-water-a review. *Journal of*
40
41 429 *Environmental Quality*, 1993, 22:392-402.
42
43
44
45 430 Stein, H.; Kellermann, C.; Schmidt, S.I.; Brielmann, H.; Steube, C.; Berkhoff, S.E.; Fuchs,
46
47 431 A.; Hahn, H.J.; Thulin, B.; Griebler, C. The potential use of fauna and bacteria as ecological
48
49 432 indicators for assessment of groundwater quality. *Journal of Environmental Monitoring*,
50
51 433 2010, 12:242-254.
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 434 Vitòria, L. Estudi multi-istòpic (^{15}N , ^{34}S , ^{13}C , ^{18}O , D, $^{87}\text{Sr}/^{86}\text{Sr}$) de les aigües
2
3 435 subterrànies contaminades per nitrats d'origen agrícola i ramader. PhD dissertation.
4
5 436 Universitat de Barcelona, 2004.
6
7
8 437 Vitòria, L.; Soler, A.; Canals, A.; Otero, N. Environmental isotopes (N, S, C, O, D) to
9
10 438 determine natural attenuation processes in nitrate contaminated waters: example of Osona
11
12
13 439 (NE Spain). *Applied Geochemistry*, 2008, 23:3597–3611.
14
15
16 440
17
18
19 441
20
21
22
23 442
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

443 **FIGURE CAPTIONS**

1
2 444 **Figure 1:** Geographical and geological setting of the distinct study areas.

3
4 445 **Figure 2.** Box plots of the main hydrochemical parameters according to the aquifer groups
5
6
7 446 and nitrate concentrations ranges: Low nitrate concentrations refer to values below 50 mg/L,
8
9 447 and high nitrate concentrations refer to values above 50 mg/L (see SI2 for details). Legend:
10
11 448 Boxes represent the 25 and 75 percentiles and the median; while a white line represents the
12
13 449 mean value. Bars define the 10 and 90% percentile, and dots refer to extreme values.

14
15
16 450 **Figure 3.** PCA scores distribution of the different samples, considering denitrification,
17
18
19 451 plotted according the lithological group and the $\delta^{15}\text{N}_{\text{NO}_3}$, content: a) VF1 vs VF2, b) VF3 vs
20
21 VF2, and c) VF4 vs VF2.

22 452
23
24 453 **Figure 4.** Bivariate relationships of SO_4^{2-} , Ca^{2+} and Cl^- vs. NO_3^- . Linear regression equations
25
26 454 are calculated for all the samples of the group, disregarding their $\delta^{15}\text{N}$ value. *Legend:* red
27
28 circles, samples with $\delta^{15}\text{N} > 15\text{‰}$; black circles, samples with $\delta^{15}\text{N} < 15\text{‰}$; white circles,
29
30 455 samples with $\delta^{15}\text{N}$ data not available.
31
32 456

33
34 457

35
36
37 458
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

459 TABLES

460 **Table 1:** Mean values and standard errors of the main physicochemical characteristics of the
 461 different aquifer groups, differentiating samples of low and high nitrate content.

	G1- Igneous and metamorphic rocks		G2- Sedimentary rocks		G3- Aquifers derived from igneous and metamorphic rocks		G4- Aquifers derived from sedimentary rocks		G5- Volcanic materials	
	<50 mg/L	>50 mg/L	<50 mg/L	>50 mg/L	<50 mg/L	>50 mg/L	<50 mg/L	>50 mg/L	<50 mg/L	>50 mg/L
Number of wells	9	4	28	36	15	27	22	29	29	5
EC ($\mu\text{S}/\text{cm}$)	819 \pm 110	915 \pm 135	819 \pm 70	1124 \pm 65	841 \pm 72	1073 \pm 78	770 \pm 27	1070 \pm 64	623 \pm 21	779 \pm 31
pH	7.18 \pm 0.14	6.99 \pm 0.04	7.35 \pm 0.09	7.43 \pm 0.06	7.47 \pm 0.12	7.28 \pm 0.08	7.18 \pm 0.04	7.08 \pm 0.04	7.47 \pm 0.06	7.25 \pm 0.21
Eh (mV)	236.7 \pm 62.1	386.5 \pm 39.3	190.1 \pm 20.1	247.4 \pm 27.4	354.9 \pm 38.7	379.8 \pm 8.3	161.0 \pm 13.5	164.3 \pm 17.3	274.6 \pm 21.0	240.0 \pm 10.0
T ($^{\circ}\text{C}$)	17.4 \pm 0.5	16.9 \pm 0.3	15.4 \pm 0.6	15.9 \pm 0.4	16.3 \pm 0.4	16.4 \pm 0.2	15.1 \pm 0.4	13.2 \pm 0.2	14.5 \pm 0.6	14.5 \pm 0.4
O ₂ (mg/L)	1.5 \pm 0.6	3.9 \pm 1.4	4.0 \pm 0.6	4.0 \pm 0.5	3.7 \pm 1.1	5.2 \pm 0.5	4.6 \pm 0.6	4.4 \pm 0.4	8.0 \pm 0.6	7.6 \pm 1.3
HCO ₃ ⁻ (mg/L)	368.1 \pm 59.6	346.9 \pm 40.0	418.3 \pm 15.4	388.0 \pm 13.0	355.1 \pm 34.4	360.7 \pm 13.5	411.9 \pm 18.6	407.4 \pm 13.1	284.8 \pm 13.0	341.7 \pm 39.0
Cl ⁻ (mg/L)	101.0 \pm 24.7	99.2 \pm 42.8	42.4 \pm 6.7	94.9 \pm 10.4	86.1 \pm 15.1	91.8 \pm 11.9	16.0 \pm 1.6	73.3 \pm 12.4	16.1 \pm 2.2	30.7 \pm 10.4
SO ₄ ²⁻ (mg/L)	35.9 \pm 5.7	57.2 \pm 8.6	132.4 \pm 46.4	164.3 \pm 32.1	68.4 \pm 16.8	98.1 \pm 12.4	43.5 \pm 5.4	184.6 \pm 34.6	48.0 \pm 6.5	41.4 \pm 8.2
NO ₃ ⁻ (mg/L)	13.7 \pm 4.2	105.3 \pm 20.3	17.0 \pm 2.5	166.3 \pm 19.4	18.6 \pm 4.4	137.5 \pm 16.6	25.4 \pm 2.7	172.2 \pm 25.2	23.9 \pm 2.4	71.4 \pm 10.0
Na ⁺ (mg/L)	104.2 \pm 38.0	79.7 \pm 18.6	34.8 \pm 4.2	54.3 \pm 7.5	63.5 \pm 12.0	56.4 \pm 5.1	12.5 \pm 1.4	42.0 \pm 5.6	16.5 \pm 1.5	21.5 \pm 9
K ⁺ (mg/L)	2.2 \pm 0.4	1.0 \pm 0.3	4.4 \pm 1.7	8.5 \pm 2.4	2.4 \pm 0.4	5.8 \pm 3.0	2.8 \pm 0.6	8.8 \pm 2.1	7.6 \pm 0.7	6.9 \pm 1.7
Ca ²⁺ (mg/L)	82.6 \pm 14.0	127.5 \pm 21.5	132.6 \pm 16.2	185.7 \pm 10.1	110.7 \pm 12.7	166.3 \pm 9.7	135.8 \pm 7.1	196.0 \pm 10.8	87.2 \pm 4.5	89.5 \pm 8.4
Mg ²⁺ (mg/L)	14.6 \pm 3.6	22.0 \pm 3.5	37.4 \pm 5.4	38.2 \pm 3.8	19.3 \pm 2.0	22.7 \pm 2.4	21.0 \pm 1.1	44.1 \pm 5.6	19.5 \pm 1.2	34.1 \pm 3.5

462

463

464

465 **Table 2.** Loadings obtained in the second PCA, including $\delta^{15}\text{N}_{\text{NO}_3}$, among the different
 466 parameters.

	VF1	VF2	VF3	VF4
SO_4^{2-} (mg/L)	<u>0.965</u>	0.008	-0.005	0.039
Mg^{2+} (mg/L)	<u>0.885</u>	0.181	0.007	0.249
Ca^{2+} (mg/L)	<u>0.740</u>	<u>0.562</u>	0.004	0.035
EC ($\mu\text{S}/\text{cm}$)	<u>0.641</u>	<u>0.546</u>	<u>0.425</u>	0.184
NO_3^- (mg/L)	0.121	<u>0.949</u>	0.112	0.057
$\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (‰)	-0.238	0.097	<u>0.848</u>	-0.178
Na^+ (mg/L)	0.295	0.054	<u>0.738</u>	<u>0.426</u>
Cl^- (mg/L)	0.289	<u>0.520</u>	<u>0.601</u>	<u>0.405</u>
$\delta^{15}\text{N}_{\text{NO}_3}$ (‰)	0.113	0.085	0.047	<u>0.935</u>
Eigenvalue	2.928	1.837	1.821	1.352
% Variance	32.53	20.41	20.23	15.03

467

468

469

470

Figure 1

[Click here to download high resolution image](#)

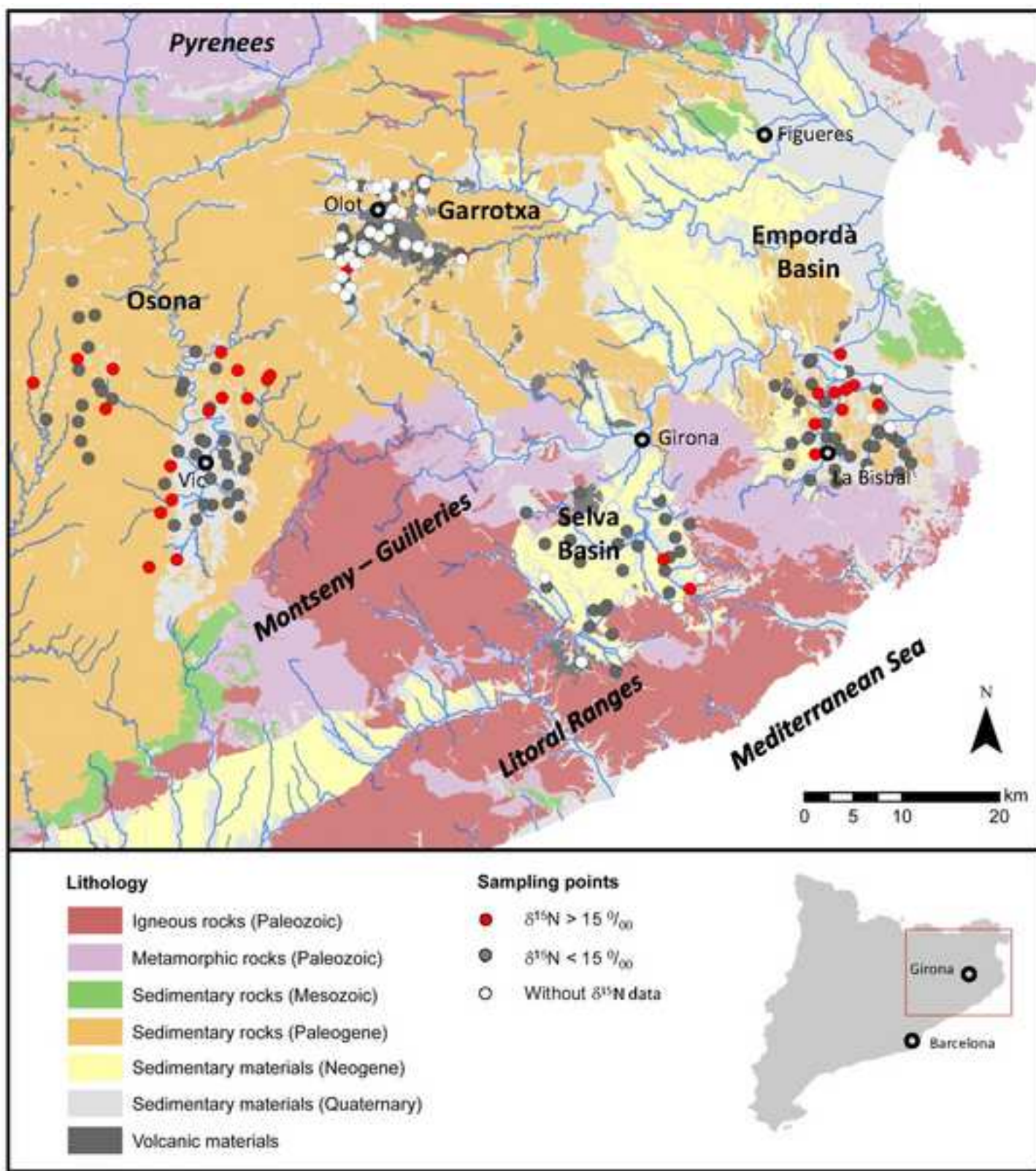


Figure 2
[Click here to download high resolution image](#)

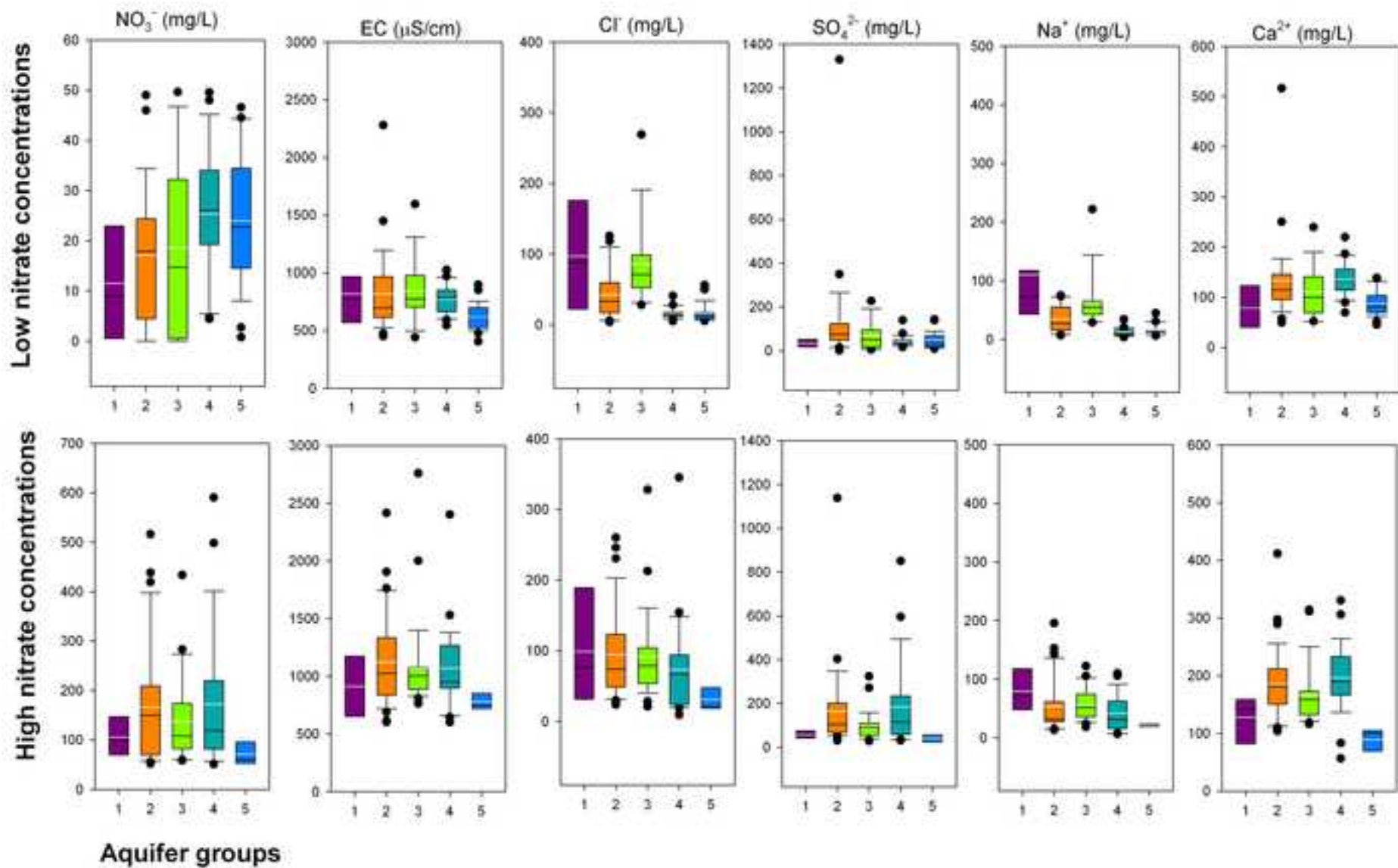


Figure 3
[Click here to download high resolution image](#)

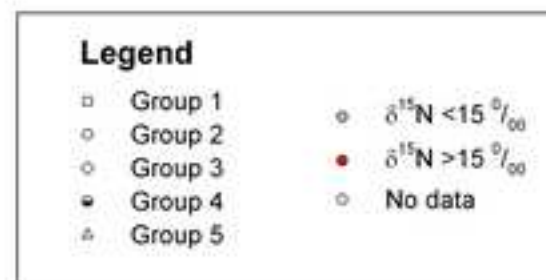
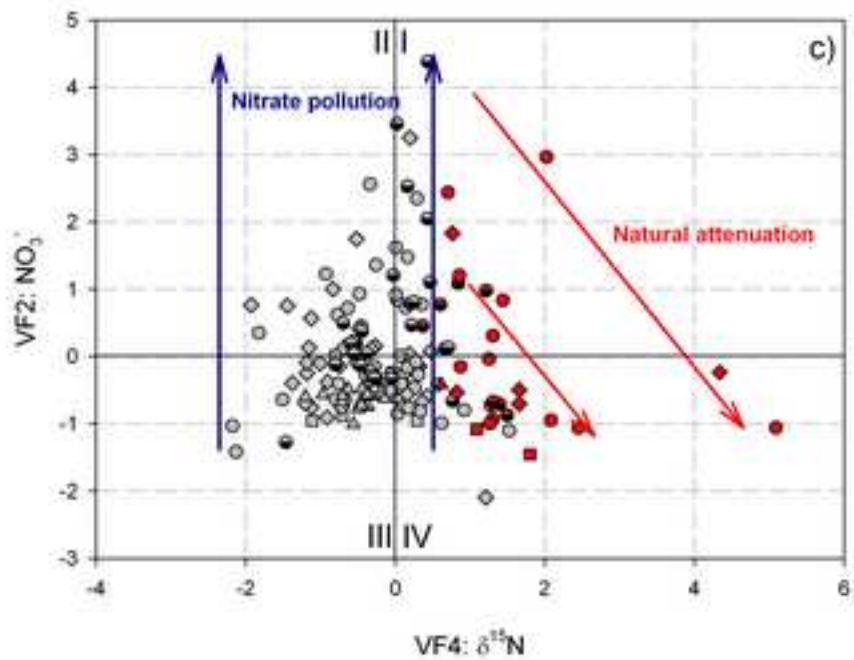
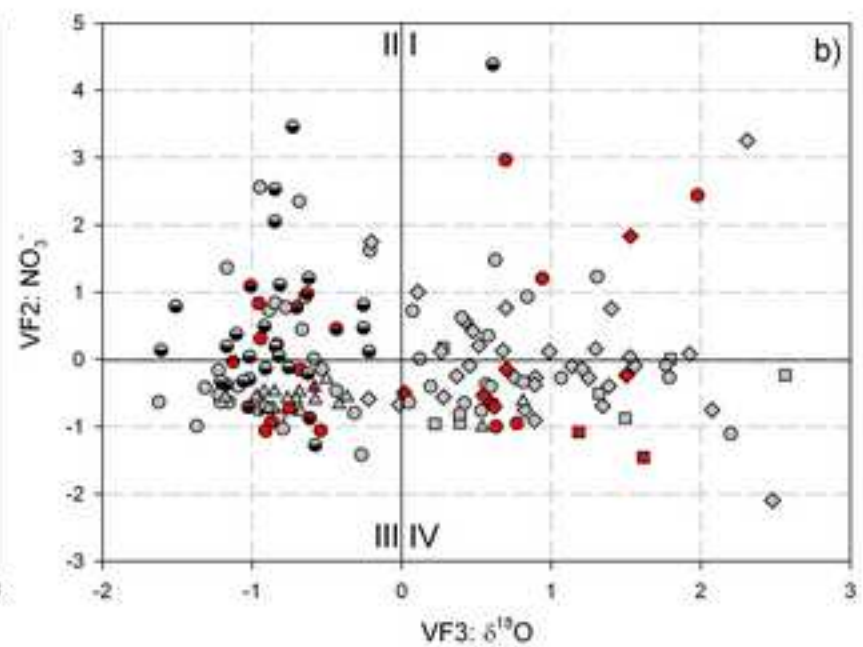
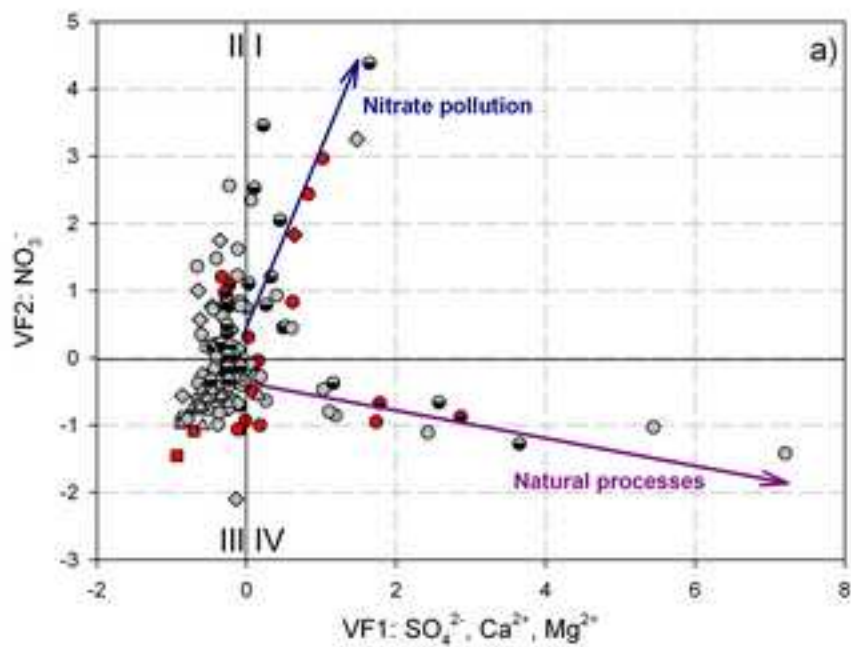


Figure 4
[Click here to download high resolution image](#)

