

Multi-isotopic and compositional exploration of factors controlling nitrate pollution

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Abstract

In Catalonia, according to the nitrate directive (91/676/EU), nine areas have been declared as vulnerable to nitrate pollution from agricultural sources (Decret 283/1998 and Decret 479/2004). Five of these areas have been studied coupling hydro chemical data with a multi-isotopic approach (Vitòria et al. 2005, Otero et al. 2007, Puig et al. 2007), in an ongoing research project looking for an integrated application of classical hydrochemistry data, with a comprehensive isotopic characterisation ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of dissolved nitrate, $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ of dissolved sulphate, $\delta^{13}\text{C}$ of dissolved inorganic carbon, and δD and $\delta^{18}\text{O}$ of water). Within this general frame, the contribution presented explores compositional ways of: (i) distinguish agrochemicals and manure N pollution, (ii) quantify natural attenuation of nitrate (denitrification), and identify possible controlling factors.

To achieve this two-fold goal, the following techniques have been used. Separate biplots of each suite of data show that each studied region has a distinct $\delta^{34}\text{S}$ and pH signatures, but they are homogeneous with regard to NO_3^- related variables. Also, the geochemical variables were projected onto the compositional directions associated with the possible denitrification reactions in each region. The resulting balances can be plot together with some isotopes, to assess their likelihood of occurrence.

Key words: isotope, geochemistry, nitrate pollution, pig manure, compositional data

1 Introduction

In the last decades, nitrate pollution has become a major threat to groundwater quality, as the threshold value for drinking water (50 mg/l, Directive 98/83/EC) is achieved in most of the local and regional aquifers in Europe. High nitrate in drinking water poses a health risk, because the ingestion of high nitrate concentration can cause methaemoglobinaemia in children and babies (Magee and Barnes, 1956), and some authors pointed out that nitrogen compounds can act as human cancer promoters (Ward et al., 2005; Volkmer et al., 2005). Nitrate pollution is linked to the intensive use of synthetic and organic fertilizers, as well as to septic systems effluents. In Catalonia, according to the nitrate directive (91/676/EU), nine areas have been declared as vulnerable to nitrate pollution from agricultural sources (Decret 283/1998 and Decret 479/2004), and 307 municipalities, one third of the Catalonia municipalities, are located in these areas.

To improve water management in these areas, it is essential to determine the origin of pollution and the evolution of nitrogen compounds. Nitrate isotopes are a unique tool for this purpose. Nitrogen and oxygen isotopes of dissolved nitrate can be used as tracers of nitrate pollution, distinguishing between chemical fertilizers, and manure or sewage (Wassenaar, L. 1995; Kendall and Mc Donnell, 1998, among others). However, in order to use the isotopic composition of dissolved nitrate as a tracer of nitrate origin, one must bear in mind that several processes (e.g. volatilization, nitrification and denitrification) change the isotopic composition of the sources, leading to overlapping isotopic signatures for different nitrate sources. This fact, considered a drawback in the application of nitrate isotopes as tracers of sources, can be applied to trace processes themselves, e.g. the isotopic composition of dissolved nitrate is used to distinguish between dilution and denitrification in groundwater samples in areas where a diminution in nitrate concentration is observed (Griseck et al, 1998; Cey et al, 1999; Mengis et al. 1999). A further step in the investigation of denitrification processes is to determine the factors controlling the reaction, this has been done coupling chemical data with the $\delta^{15}\text{N}$ and/or $\delta^{18}\text{O}$ of dissolved nitrate and the isotopic composition of the ions involved in denitrification reactions, as $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ of dissolved sulphate, and/or $\delta^{13}\text{C}$, of dissolved inorganic carbon (Aravena and Robertson, 1998, Pauwels et al. 2000).

This approach was proposed in an ongoing project performed in several areas classified as vulnerable to nitrate pollution in Catalunya. Five of the vulnerable areas (Empordà, Garrotxa, Maresme, Osona, and Selva) have been studied coupling classical hydrochemistry data with a comprehensive isotopic characterisation, including $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of dissolved nitrate, $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ of dissolved sulphate, $\delta^{13}\text{C}$ of dissolved inorganic carbon, and δD and $\delta^{18}\text{O}$ of water, (Vitòria et al. 2005; Otero et al. 2007; Puig et al. 2007). The key goals of this project were (i) to identify the main sources of nitrate pollution in the areas, (ii) to verify if denitrification (natural attenuation) processes were taking place, and (iii) to determine the factors controlling the denitrification reactions.

2 Study area

The studied areas are located in Barcelona and Girona provinces, 4 of them correspond to inner basins and one is located in the coast (Fig. 1). The Ter River is a major stream in the area, it crosses the Osona, Selva and Empordà studied areas. Its mean discharge in the Osona area is 509 hm³/year (at the gauging station of Roda de Ter), in the Selva area 261 hm³/year (at the gauging station of Cellera de Ter), and in the Empordà area 1026 hm³/year (at the gauging station of Colomers). The second major stream in the area is the Fluvià River, which crosses the Garrotxa area and its mean discharge is 23 hm³/year (at the gauging station of Olot). The studied areas have a sub-Mediterranean climate with mean rainfall between 550 and 850 mm/year for all the areas, except Garrotxa, with higher pluviometry (900 to 1150 mm/year). The potential evapotranspiration is in the range of rainfall (570 to 850 mm/year). The different areas are characterised by different geology, hydrogeology and land uses.

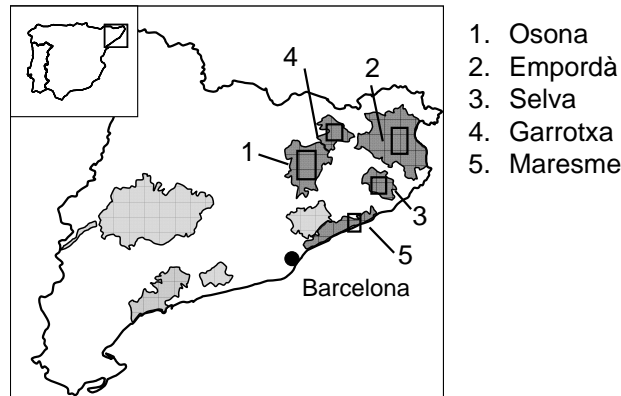


Figure 1: Map of Catalunya showing the areas classified as vulnerable to nitrate pollution from agricultural sources. The darker grey indicates the areas studied.

2.1 Geology

The Osona area, from a geological perspective, is constituted of Paleogene sedimentary materials overlaying hercynian crystalline (igneous and metamorphic) rocks. The stratigraphic sequence primarily consists of carbonate formations, with an alternation of calcareous, marl and carbonate sandstone layers. It is worth noting the presence of disseminated pyrite in marls. These formations show a quite uniform dipping of about 7-10° to the west. The Empordà area consists of Paleogene detritic and carbonate sedimentary rocks (east and south), Neogene clay facies (west), and Holocene alluvial deposits of Ter River, the main stream in the area, and its tributary Daró. These Tertiary and Quaternary materials lay on Paleozoic discordant bedrock which outcrops in the western and southern parts of the watershed. The geology of the Maresme area is quite different, it consists of Holocene alluvial deposits of coarse sands derived from the weathered granodiorite that forms the Catalan Coastal Range. The Selva area belongs to a tectonic basin surrounded by three 1000 m asl ranges. This basin was created during the distensive periods after the Alpine orogenesis, and it has a Neogene sedimentary poorly consolidated filling. Surrounding ranges consist of Paleozoic igneous and metamorphic rocks, and pre-Alpine Paleogene sedimentary rocks (mainly limestone and sandstone). The Garrotxa area is constituted of Paleogene sedimentary formations overlaying Paleozoic basement. It is surrounded by two ranges (north and south), and Ebre and Empordà depressions (west and east, respectively). Two main faults oriented NW-SE and W-E delimit alternative horsts and grabens between the two surrounding depressions. Quaternary formations are characterized by volcanic activity materials overlaying Paleogene materials and detritic alluvial deposits.

2.2 Hydrogeology

The Osona area, hydrogeologically, is constituted by a series of confined aquifers located in the carbonate and carbonate-sandstone layers, whose porosity is mainly related to the fracture network. Marl stratum act as confining layers. Main production wells for agriculture and farm demand usually reach depths of more than 100 m, searching for the most productive confined aquifers. Alluvial aquifers are scarce and generally non-productive in the area; except those located at the Ter river terraces. The Empordà area, because of a high lithological diversity, presents a complex distribution of hydrogeological units. The basic units are: 1) an unconfined aquifer with sand and gravel, and some clay in the matrix (mainly from Quaternary) and 2) a confined and sometimes unconfined fractured aquifer with thickness discontinuity (mainly from Tertiary). The Maresme area main hydrogeological units are an unconfined sandy aquifer underlayed by an aquitard composed of silts and clays and a confined sandy aquifer. The thickness of these units is 5-40 m, 5-15 m and 15-20 m, respectively. The unconfined aquifer is the only one affected by groundwater extractions and its water table varies between 4 and 30 m in depth. In the Selva area we can differentiate a regional and a local flow system. Thus, four geological domains can hydrogeologically be distinguished: 1) the granitic materials of the surrounding ranges, which act as the main recharge area of the granitic basement of the depression; 2) the Neogene materials of the sedimentary basin, whose local flows originate from the range areas as a lateral recharge, and from the uppermost parts of the basement; 3) the main faults oriented NW-SE and NE-SW behaving as an independent hydrogeological unit connected with the rest of units (fractures responsible for the thermal springs occurring within the basin); and 4) the upper alluvial formations with two alluvial aquifers associated to two streams. The Garrotxa area presents two main hydrogeological units: 1) alluvial and

colluvial aquifers located in the Fluvia basin composed of silts and sands, with high permeability gravel, and 2) fluviovolcanic aquifers with interspersed volcanic and alluvial materials, and permeabilities depending on the nature of volcanic materials.

2.3 Land uses

The Maresme area is an intensive agricultural area. Flowers, fruit and vegetable crops are the main agricultural products, where about half of them grow under greenhouse conditions. Fertilisation is carried out with inorganic fertilizers usually injected through trickle irrigation systems that use groundwater extracted from partially-penetrating wells (5-40 m deep). The soil type in this area is usually coarse sand with a low organic matter content (<3% of dry soil) and a low C:N ratio of approximately 1:2 (Guimerà et al. 1995). This low natural fertility, together with the low water-holding capacity of the soil, requires high fertiliser and irrigation applications, resulting in high nutrient leaching in the upper zone of the aquifer affected by groundwater withdrawals. Re-circulation of the shallow groundwater by the irrigation system causes high concentrations of nitrates (up to 300 mg/l) in the groundwater. In this area only chemical fertilizers are used, therefore it is considered as representative of nitrate pollution from inorganic fertilizers.

In the central part of Osona nitrate pollution is widely extended, with a median concentration above 100 mg/l during the last five years. In this region, of 1263.8 km², there are more than 1000 pig farms, with 990000 pigs, 110000 cows and 67000 sheep. This intensive farming activity produces huge amounts of organic residues, 10900 tones of N/year. Fertilizers are also applied, especially at the surroundings of the villages, as the use of pig manure close to urban areas is forbidden. A 93% of the municipalities are connected to sewage network; therefore the contribution of sewage to groundwater nitrate pollution is expected to have negligible influence, compared to agricultural sources. In this area, although chemical fertilizers are also applied, the main contribution to nitrate pollution is linked to manure application as fertiliser or in uncontrolled dumps, hence this area is considered as representative of manure nitrate pollution.

In Empordà area there is a notable agriculture activity (mainly maize, sunflower and fruit crops) which uses synthetic fertilizers, and whose water demand for irrigation is remarkably increased in summer months. Organic fertilizers are also applied as a consequence of an intensive pig farming activity (462 pigs/Km²) which produces large amounts of organic residues. Thus, Empordà area is considered a “mixed” area where both fertilizers and animal manure are used. The other two areas, Selva and Garrotxa, can also be considered mixed areas.

3 Processes affecting the isotopic composition of N in groundwater

In order for isotopes to be useful as tracers of N sources, it is important to understand how biogeochemical cycling affects the isotopic composition of various N-bearing compounds (Kendall et al., 2007). In groundwater studies the major biogeochemical processes that can modify the $\delta^{15}\text{N}$ of sources are especially ammonia volatilization, nitrification, and denitrification.

3.1 Volatilization

Volatilization is a physical process that consists of the loss of ammonia gas to the atmosphere, and it results in a high increase of the $\delta^{15}\text{N}$ residual ammonium (Letolle, 1980). Ammonium produced from applications of urea and manure to fields may have $\delta^{15}\text{N}$ values >20‰ due these ammonia losses.

3.2 Nitrification

Nitrification is the two-step process of NH_4^+ oxidation to NO_3^- mediated by several different autotrophic bacteria for the purpose of deriving metabolic energy:



In systems where nitrogen is the limitant factor and the reaction goes to completion, i.e. NH_4^+ is rapidly converted to NO_3^- , the $\delta^{15}\text{N}$ of the nitrate produced is the same to the $\delta^{15}\text{N}$ of the reactant ammonium. On the other hand, the oxidant sources

that control the $\delta^{18}\text{O}$ resulting from nitrification are the ambient H_2O and O_2 . Thus, the $\delta^{18}\text{O}_{\text{NO}_3}$ is interpreted as a mixture of two oxygen atoms from H_2O and one from O_2 :

$$\delta^{18}\text{O}_{\text{NO}_3} = 2/3(\delta^{18}\text{O}_{\text{H}_2\text{O}}) + 1/3(\delta^{18}\text{O}_{\text{O}_2})$$

where $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ is assumed to be groundwater oxygen isotopic composition, and $\delta^{18}\text{O}_{\text{O}_2}$ the $\delta^{18}\text{O}$ of atmospheric O_2 (+23.5‰).

3.3 Denitrification

In denitrification processes NO_3^- is reduced to gaseous products like N_2 , N_2O or NO , and usually requires anoxic conditions (less than 20 μM of O_2). There are two reaction pathways that involve nitrate reduction: heterotrophic denitrification, where nitrate is reduced by heterotrophic microbes while organic matter oxidation occurs (Eq. 1), and chemo-autotrophic denitrification by bacteria such as *Thiobacillus denitrificans* which oxidizes sulphur, e.g. in pyrites (Eq. 2):



In both cases, a decrease in nitrate concentration is coupled with an increase in the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of residual nitrate with a $\epsilon\text{N}/\epsilon\text{O}$ ratio that ranges from 1.3 (Fukada et al., 2003) to 2.1 (Böttcher et al., 1990).

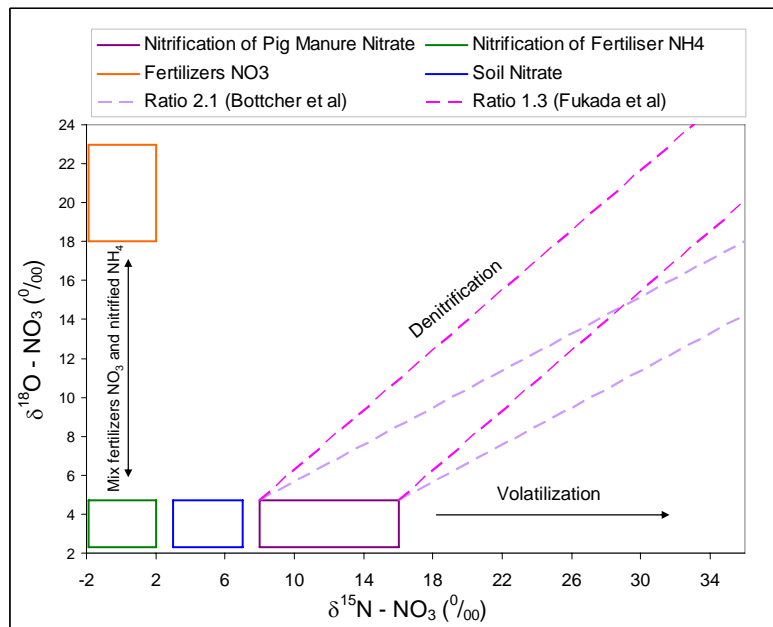


Figure 2: Isotopic values of the main nitrogen sources in the studied area (Vitória et al. 2004a, 2004b, Kendall et al. 2007), the arrows indicate the evolution of the isotopic composition considering volatilization and mixing processes. Dashed lines are the expected denitrification trends following the enrichment factors from the literature, and taking as departure point the minimum and maximum isotopic composition of nitrate from nitrification of organic manure.

4 Methods

4.1 Analytical methods

All the sampling surveys were conducted on production wells. In the Osona area different sampling surveys were conducted. The first in a reduced area of 36 km² with 38 samples. The second, in the same small area, where a monthly sampling of 6 sites throughout one year was conducted. Three more surveys were conducted in a larger area 600 km², with 59, 58 and 32 samples respectively. In the Empordà area two surveys were conducted in a 200 km² area. The first survey of 24 samples and the second of 40 samples. In the Maresme area two sampling surveys, in a small area (3 km²) area were conducted, the first of 8 samples and the second of 23 samples. In both the Selva and Garrotxa areas only one survey was conducted, with 38 samples in a 350 Km² area in Selva zone, and 31 samples in a 100 Km² area in Garrotxa zone. Physicochemical parameters (pH, temperature, conductivity, dissolved O₂ and Eh) were measured in situ, using a flow cell to avoid contact with the atmosphere. Eh was measured with an Orion model 96-78 platinum–calomel electrode pair connected to an Oakton 35615 series millivolt meter. All Eh readings were corrected to the normal hydrogen electrode potential by adding the standard electrode potential at the groundwater temperature. Samples were collected after wells had been continuously pumped until Eh values became stabilised. Samples were stored at 4°C and dark environment. Chemical parameters were determined by standard analytical techniques, the chemical characterisation comprises major ions (Cl⁻, SO₄²⁻, HCO₃⁻, Na⁺, Ca²⁺, Mg²⁺, K⁺), nitrogen compounds (NO₂⁻; NO₃⁻, NH₄⁺), Fe and Mn. The δD and δ¹⁸O of water were obtained by H₂ and CO₂ equilibrium, respectively, and Isotope Ratio Mass Spectrometry (IRMS) with a Delta S Finnigan Mat. Nitrogen and oxygen isotopes of dissolved nitrate For δ¹⁵N_{NO₃} and δ¹⁸O_{NO₃} analysis, dissolved nitrates were concentrated using anion-exchange columns Bio Rad® AG 1-X8(Cl⁻) 100-200 mesh resin, after extracting sulphates and phosphates by precipitation with BaCl₂ and filtration (Mayer et al., 2001). Afterwards dissolved nitrates were eluted with HCl and converted to AgNO₃ by the addition of silver oxide. The silver nitrate solution was then freeze-dried obtaining the pure AgNO₃ for analysis (modified from Silva et al. 2000). Two surveys of the Osona area were analysed for nitrogen and oxygen isotopes of dissolved nitrate following the methods of Sigman et al. (2001) and Casciotti et al. (2002). For sulphur and oxygen isotopic analysis, the dissolved sulphate was precipitated as BaSO₄ by the addition of BaCl₂•2H₂O, after acidifying the sample with HCl and boiling it. For δ¹³C analysis unfiltered splits of samples were treated with NaOH-BaCl₂ solution to precipitate carbonates, and then filtered at 3 μm. The sulphur, nitrogen and carbon isotopic composition was determined with an Elemental Analyser (Carlo Erba 1108) coupled with an IRMS (Delta C Finnigan Mat). The oxygen isotopic composition of nitrate and sulphate was analysed in duplicate with a Thermo-Chemical Elemental Analyser (TC/EA Thermo-Quest Finnigan) coupled with an IRMS (Delta C Finnigan Mat). Notation is expressed in terms of δ per mil relative to the Vienna Standard Mean Ocean Water (V-SMOW), atmospheric N₂ (AIR), Vienna Canyon Diablo Troilite (V-CDT), and Vienna Peedee Belemnite (V-PDB) standards. The isotope ratios were calculated using international and internal laboratory standards. Reproducibility Precision (≡1σ) of the samples calculated from standards systematically interspersed in the analytical batches is ±1.5‰ for δD, ±0.2‰ for δ¹⁸O_{H₂O}, ±0.3‰ for δ¹⁵N_{NO₃}, ±0.2‰ for δ³⁴S, ±0.5‰ for both δ¹⁸O_{NO₃}, and δ¹⁸O_{SO₄}, and ±0.2‰ for δ¹³C_{HCO₃}. Isotopic analyses were prepared at the laboratory of the Mineralogia Aplicada i Medi Ambient research group and analysed at the Serveis Científicotècnics of the Universitat de Barcelona, except the isotopic composition of dissolved nitrates of two surveys in the Osona area, analyzed at the Woods Hole Oceanographic Institution.

4.2 Statistical methods

The statistical analysis of the several variables is conducted attending to the characteristics of each data type.

- The isotope data can be considered to be unbounded linearly independent variables (from an algebraic point of view), as they represent (signed) departures from a central value and the set of all isotopes considered is by far not closed. Moreover, Tolosana et al. (2005) showed that isotopic delta values represent first order Taylor approximations to the log-ratios of the two isotopes involved in a delta. Given that isotopic data vary almost always in a very small range, these approximations are almost always extremely good: as a result, one can consider an isotope delta as a linear function of the log-ratio of the two isotopes involved
- Acidity measured through pH is in fact expressed in a logarithmic scale (decimal logarithm, with changed sign). Similarly, Eh potential is already an unbounded quantity with an additive scale, representing additive increments with respect to Hydrogen Eh value. These two variables will be therefore kept as they are.
- The major components (from HCO₃ to TOC) will enter the analysis in two ways: in logarithms (positive scale, Fig. 2) and in log-ratios (compositional scale). The minor components (Mn and Fe) will be considered as binary (above/below the detection limit) or log-transformed.

The first step will be the discrimination of the 5 sampled zones by means of the delta data set, of the geochemistry (in log-scale), and of both data sets mixed. Linear discriminant analysis will look for the 4 linear combinations of the available variables such that the means of the groups are at a maximum distance from each other, measured in Mahalanobis distance on this 4-dimensional space, thus accounting for the variances and correlations between variables. The coefficients of these linear combinations will be expressed in a sort of covariance biplot, by scaling them with the standard deviations of both the discriminant functions and the data variables. This pursues the representation of the associations between variables and groups, not of the discriminant power of each variable (which is given by the unscaled coefficient itself).

The other analysis is the selection of some adequate projections, following the two hypothesis reactions, namely pyrite oxidation or organic matter oxidation. Given the reaction equations described in section 3.3 one should expect that the following log-ratios increase with increasing oxidation,

$$I_{MO} = 4 \ln \frac{HCO_3}{NO_3} \quad I_P = \ln \frac{(SO_4)^{10}}{(NO_3)^{14} \cdot (H)^4} \quad (\text{Eq. 3})$$

if the system was closed, which is rather improbable. Nevertheless, we know that both reactions should modify the isotopic composition of the $\delta^{15}N$ and $\delta^{18}O$ of NO_3 (towards higher isotopic composition), and that each reaction should may also change the isotopic composition of the ions involved, $\delta^{13}C$ of dissolved inorganic carbon (towards lower values) and $\delta^{34}S$ and $\delta^{18}O$ of SO_4 (towards lower values), respectively. Thus:

- under organic matter oxidation, we may expect positive correlations between the variables I_{MO} , $\delta^{15}N$ and $\delta^{18}O_{NO_3}$, and negative correlations between those and $\delta^{13}C$;
- under pyrite oxidation, we may expect positive correlations inside the groups of variables $\{I_P, \delta^{15}N \text{ and } \delta^{18}O_{NO_3}\}$ and $\{\delta^{34}S \text{ and } \delta^{18}O_{SO_4}\}$, and negative correlations among the two groups.

Thus, a look at these selected correlations should at least allow us to reject that these processes are strongly occurring, if these conditions are not met.

5 Results and discussion

5.1 Box-plots: geochemistry and isotopes comparison

The resemblances and differences between the areas in terms of chemical components and isotopic compositions are presented in box-plots (Fig. 3). The comparison of every studied zone with another has to be done taking into account the similar or different geological backgrounds, the possible sources of pollution (pig manure, chemical fertilizers or the local lithology) and the actual processes undergone by the elements involved in.

Median pH values range between 6.9 and 7.8, with Empordà area presenting the higher values and Selva area the lower. In the latter area low pH values could be caused by the influence of the CO_2 -rich fluids within the deep levels of the basin. Eh and HCO_3 do not present important differences between the areas, though low Eh values (around 200 mV) of few samples in Empordà and Osona must be considered when redox conditions are assessed. The slightly higher HCO_3 median of Osona samples can be attributed to its carbonated aquifers. On the other hand, the uniform HCO_3 data set may be linked to $\delta^{13}C$ -DIC data set, whose range is quite constant in all the areas as a consequence of the buffered carbonate system. As NO_3 concentration indicates neither the sources nor the processes but the amount of pollution, the five areas can be arranged by degree of contamination from the more polluted to the less polluted one: Maresme, Osona, Empordà, Selva and Garrotxa. The same pattern of relative differences between the medians of every zone occurs with SO_4 , Cl, Ca and Mg concentrations, which indicates an anthropogenic contribution of one or more sources. Apart from being mainly made by N, P and K, fertilizers have also high contents of S (up to 20%), Mg, Ca, Cl (1% to 10%), and low Na contents (<0.3%) (Otero et al., 2005). Moreover, pig manure contains nitrogen in the reduced form (NH_4^+ contents up to 4000 mg/l) or as organic nitrogen, but also other major elements as Cl, K (4000 mg/l) and Na (1000 mg/l) (Mg and Ca tend to be rapidly absorbed to the solid phase). Therefore, the use of synthetic fertilizers can account for SO_4 , Ca and Mg concentrations, although, for instance, evaporites dissolution in Garrotxa area cannot be discarded as another SO_4 contribution. Regarding Na concentrations, Maresme and Selva present the higher values, possibly due to the marine intrusion and the regional flow systems fractures, respectively. The K box-plot shows Garrotxa area with a wide concentration range and the higher contents, mainly caused by volcanic materials of the zone and the synthetic fertilizers applied; K concentrations of Osona area are surely due to pig manure.

The $\delta^{15}\text{N}$ median value of Maresme is the lowest in the boxplot, and the median value of Osona is the highest. This agrees with the fact that if no processes are involved, fertilizers would have lower $\delta^{15}\text{N}$ than pig manure samples. Thus, $\delta^{15}\text{N}$ is the only parameter observed where the zone representative of fertilizers (Maresme) and the zone representative of pig manure (Osona) are the “extreme” values, and the so-called mixed areas have intermediate $\delta^{15}\text{N}$ values. The lower $\delta^{18}\text{O}_{\text{NO}_3}$ values indicate that NO_3 is derived from nitrification of ammonium from either fertilizers or pig manure. High values of this parameter would indicate the influence of either fertilizer nitrate or the occurrence of denitrification processes. Therefore the $\delta^{18}\text{O}_{\text{NO}_3}$ alone is not a valid parameter to distinguish nitrate pollution sources. Between Osona and Garrotxa areas there is an isotopic contrast: whereas Osona $\delta^{34}\text{S}$ and $\delta^{18}\text{O}_{\text{SO}_4}$ values are the lowest, Garrotxa presents the highest values of the SO_4 isotopic compositions. These differences can be explained with the presence of pyrites in the former area, and with the presence of gypsum and the use of synthetic fertilizers in the latter. Regarding water isotopes (δD and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$), the lower values observed correspond to Osona and Garrotxa areas, and the higher values are observed in Maresme, Empordà and Selva areas. The differences are due to latitude and altitude effect.

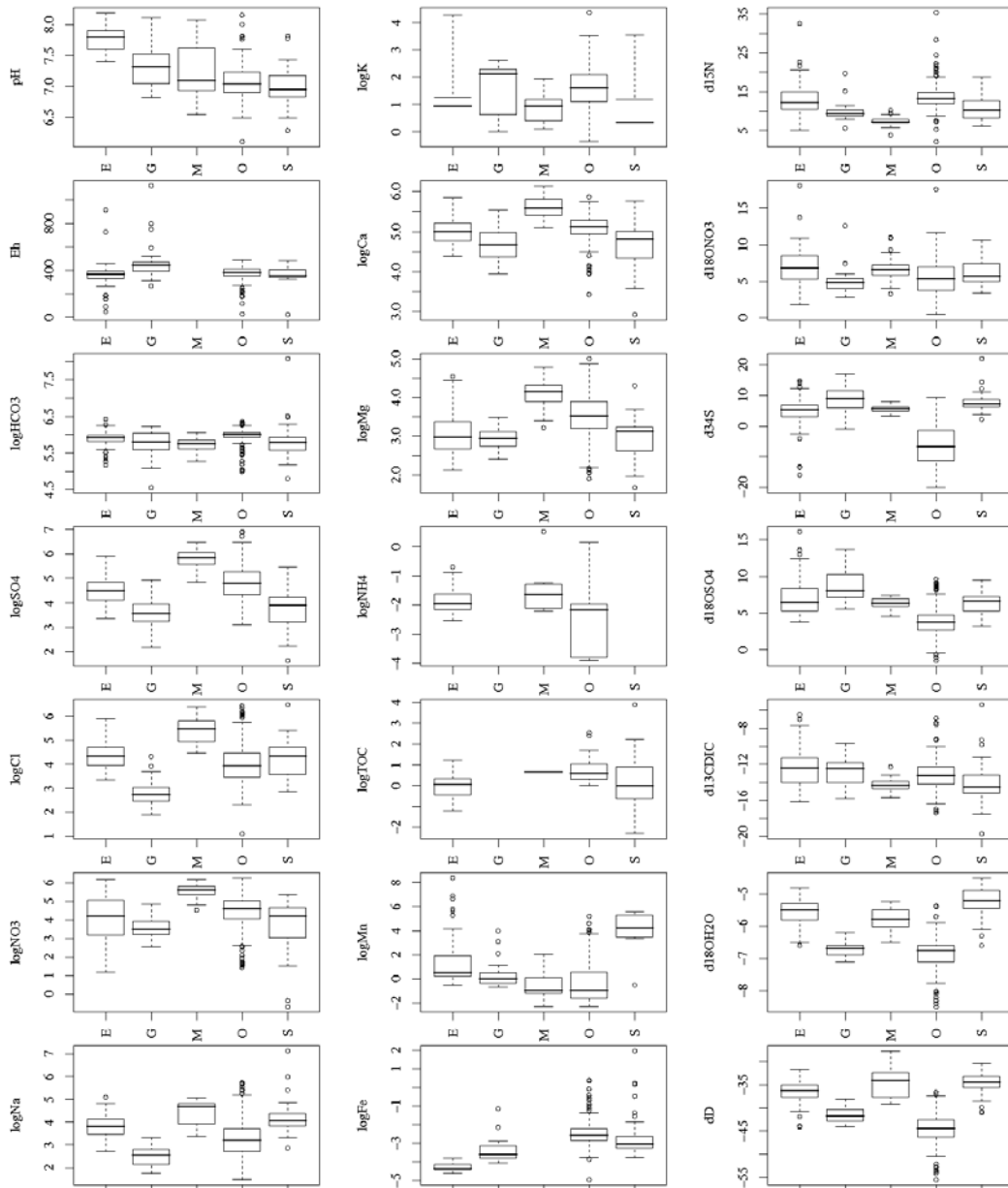


Figure 3: Box-plots of the data set split by study areas. Compositional variables were applied a logarithm transformation. Some metals were zero (below detection limit), and do not appear in the plot.

5.2 Discriminant plots: identification of pollution sources.

The representation of the association between variables and sample groups is intended to (i) discriminate the 5 sampled zones, (ii) observe which variables or combination of variables condition this discrimination, and (iii) whether the associations showed by the plot are related either to the sources of pollution (fertilizers, pig manure, or others) or to the geological background: e.g. if the main explanatory variable is *smow* (defined from δD and $\delta^{18}O_{H_2O}$) or *dsmow* (defined from the anomalies of δD and $\delta^{18}O_{H_2O}$ with regard to the meteoric water line), we are observing the effect of latitude, altitude or temperature, but it is not related to the origin of pollution. In the discriminant plots, only isotope data, only chemical data and both data sets together have been used.

In the discriminant plot using only isotopes (Fig. 4) Osona and Garrotxa samples are well separated from Maresme, Empordà and Selva samples, and the explanatory variables are *smow* and *dsmow*, indicating different latitude, altitude or continental effect. In the “link” formed by $\delta^{15}N$ and $\delta^{34}S + \delta^{18}O_{NO_3}$ (or using the difference between $\delta^{15}N$ and the sum of $\delta^{34}S$ and $\delta^{18}O_{NO_3}$), Osona and Maresme areas are the “extreme” areas: Osona with higher $\delta^{15}N$, and lower $\delta^{34}S$ and $\delta^{18}O_{NO_3}$, and the Maresme area with lower $\delta^{15}N$ and higher $\delta^{18}O_{NO_3}$. The rest of the areas are between them, confirming a mixing behavior. The difference between Osona and Garrotxa, and between Maresme and Empordà is in the direction of the $\delta^{18}O_{SO_4}$. In the case of Osona-Garrotxa, Garrotxa samples have higher $\delta^{18}O_{SO_4}$ and Osona samples lower values, related to sulphide oxidation. In the case of Empordà-Maresme, the $\delta^{18}O_{SO_4}$ in the Empordà is higher for a subset of samples, but the median $\delta^{18}O_{SO_4}$ is the same. If processes were not involved, from this discriminant plot we could relate pollution origin in the mixed areas mainly to fertilizers, with minor contribution of manure. However, we must take into account the weight of the negative $\delta^{34}S$ in the Osona samples. In these samples, nitrate source is related to organic manure and the negative $\delta^{34}S$ is linked to the presence of pyrite (and denitrification processes). In the mixed areas, as pyrite is not present in the aquifer materials, the $\delta^{34}S$ has positive values. Those samples of the mixed areas that are affected by denitrification processes will be depicted close to the Maresme samples due to the increase in $\delta^{18}O_{NO_3}$, which in this case is not “balanced” by the contribution of negative $\delta^{34}S$, and thus, difficulting the interpretation of nitrate sources in mixed areas.

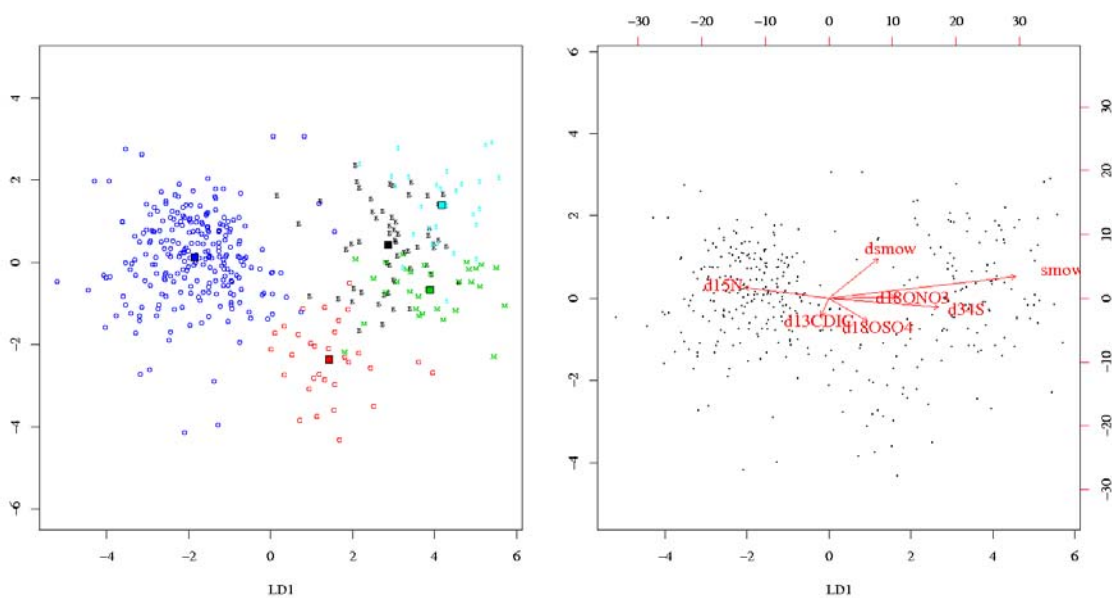


Figure 4: Discriminant plot (with indication of group means; plot centered using the global mean) and biplot (arrows for explanatory variables) using the delta data set. The missclassification rate, with a priori equally-probable groups, was 10.43 %, and the classification table was:

	E	G	M	O	S
E	41	4	3	0	10
G	2	29	0	0	0
M	1	2	28	0	0
O	2	9	1	232	0
S	4	0	3	0	22

Using only chemical data (Fig. 6) we can discriminate Selva-Garrotxa, from Empordà-Osona, and from Maresme. The explanatory variables in this case are SO_4 concentration and Na+K contents. Maresme area has the highest SO_4 values, Empordà-Osona areas have intermediate values, and Selva-Garrotxa areas are characterised by the lowest SO_4 concentration coupled with the highest Na and K contents, respectively. Furthermore, the difference between Selva and Garrotxa, and between Empordà and Osona is the ratio HCO_3^*Mg/Ca . In this case we do not have Osona and Maresme in extreme positions. The discrimination using chemical data does not allow distinguishing whether the source of pollution is linked to either fertilizers or organic manure. In this sense, it is noteworthy the weak influence of NO_3 in this discriminant plot.

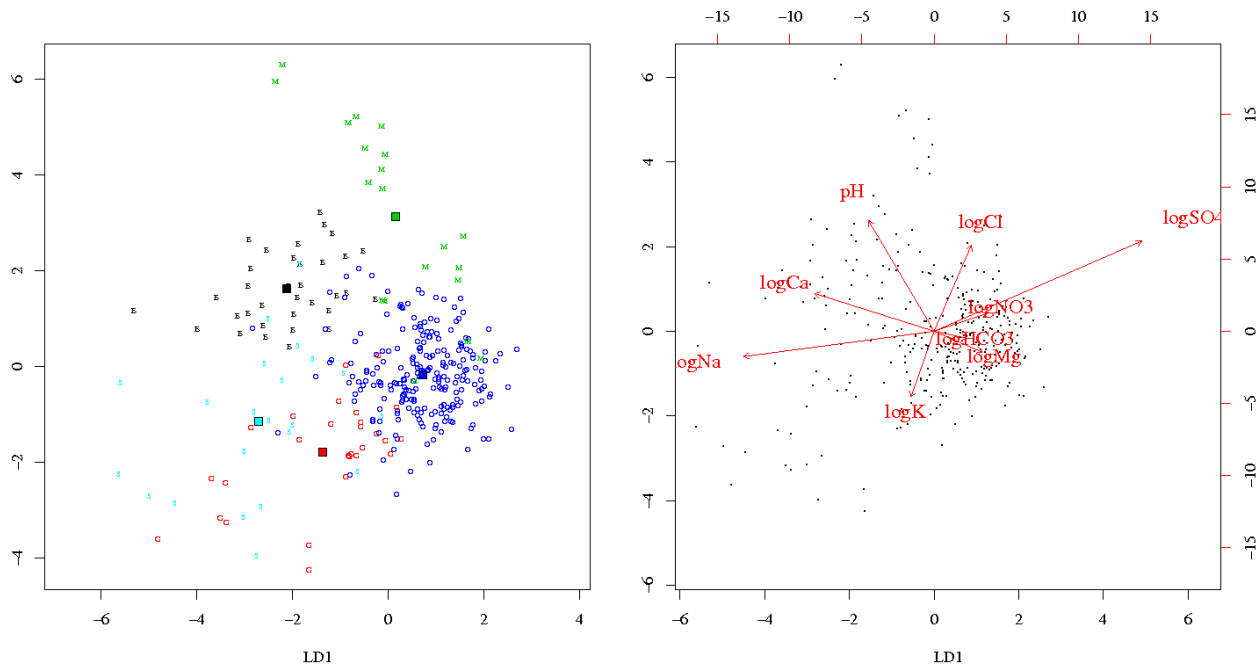


Figure 5: Discriminant plot (with indication of group means; plot centered using the global mean) and biplot (arrows for explanatory variables) using the geochemical data set. A logarithmic transformation was used, as the clr would imply the computation of the residual part, and the number of lost values distort it. Note that in the biplot, links between components still represent their log-ratio, as in a compositional representation. The missclassification rate, with a priori equally probable groups, was 12.28 %, and the classification table was:

	E	G	M	O	S
E	34	0	0	0	0
G	0	21	1	5	1
M	1	0	15	4	0
O	5	10	7	205	3
S	2	2	0	0	18

As in the first case (Fig. 5), using both data sets (Fig. 7) we can discriminate between Maresme-Selva-Empordà and Garrotxa-Osona area groups by means of the *dsmow* and *smow* variables, so this is explained by the differences in latitude, altitude and continental effect. We have a clear discrimination of samples with the ratio $\delta^{15}N/\delta^{18}O_{NO_3}$, or $\delta^{15}N*K/\delta^{18}O_{NO_3}*Cl$: Maresme samples are in one extreme and Osona samples in the opposite, the former influenced by chemical fertilizers and the latter by organic manure. Again, as in the first discriminant plot (Fig. 5), those samples from the “mixed” areas are in between. In the present plot, Selva and Empordà are closer to Maresme values (suggesting the influence of fertilizers), and Garrotxa is closer to Osona samples (indicating the influence of organic manure). We must bear in mind that in a similar direction *dsmow* and *smow* are plotted, and this variables change due to altitude and latitude effects, not linked to nitrate sources.

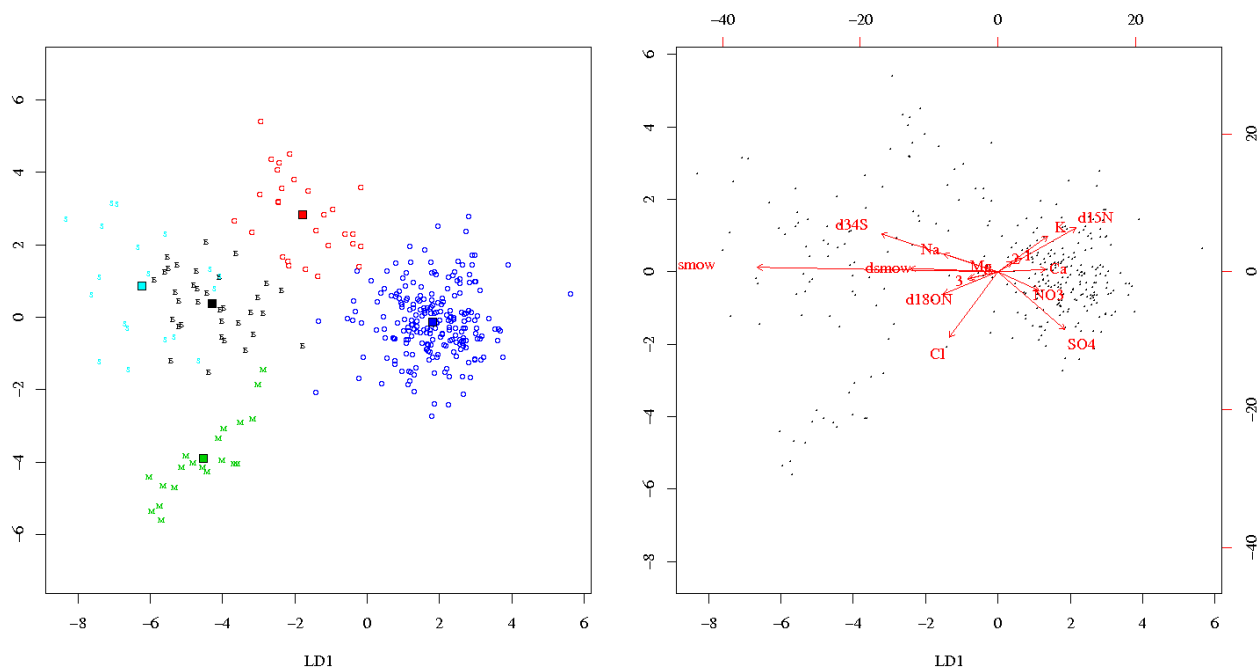


Figure 6: Discriminant plot (with indication of group means; plot centered using the global mean) and biplot (arrows for explanatory variables) using both the delta and the geochemical data set (in log scale). In the biplot, arrow 1 is $\delta^{13}\text{C-DIC}$, 2 HCO_3 and 3 $\delta^{18}\text{O}_{\text{SO}_4}$ and pH. The missclassification rate, with a priori equally probable groups, was 1.86 %, and the classification table was:

	E	G	M	O	S
E	33	0	0	0	1
G	0	27	0	1	0
M	0	0	19	0	1
O	1	2	0	219	0
S	0	0	0	0	19

5.3. Estimation of denitrification processes

One of the main objectives of this work is to determine if denitrification processes (natural attenuation of groundwater nitrate pollution) are taking place in the studied areas. As stated in section 3.3, if denitrification processes occur a coupled increase in the $\delta^{15}\text{N}$ and the $\delta^{18}\text{O}_{\text{NO}_3}$ is expected, with a $\epsilon\text{N}/\epsilon\text{O}$ ratio that ranges from 1.3 (Fukada et al., 2003) to 2.1 (Böttcher et al., 1990). In Figures 7 to 11 the first scatter plot is the $\delta^{15}\text{N}$ vs. $\delta^{18}\text{O}_{\text{NO}_3}$, and the slope determined by the ratio $\epsilon\text{N}/\epsilon\text{O}$ of the extreme enrichment factors from the literature is also depicted. These scatter plots indicate that denitrification processes are clearly taking place in Empordà and Osona areas (Fig. 7 and 10). In the Maresme area no denitrification exists, and in the Garrotxa area only two samples have isotopic values in agreement with denitrification. In the Selva area denitrification is occurring but results are not as clear as in the other areas where it does. However, discarding the occurrence of denitrification reactions based on the lack of correlation between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}_{\text{NO}_3}$, or based on a different slope of these variables, should be avoided, because in most of the areas samples are a mixing from distinct aquifer levels, and the initial NO_3 concentration and the $\delta^{15}\text{N}$ in the recharge area could differ depending on volatilization processes and rates of application. Therefore, other variables should be taken into account to assess the natural attenuation in these areas. To determine what is the main process controlling the natural attenuation (pyrite oxidation or organic matter oxidation), the two indexes calculated as described in section 4.2 were plotted against isotopic ratios of the ions involved in denitrification reactions (Figures 7 to 11).

Figure 7 shows the scatter plots of the variables related to oxidation processes in the Empordà area. In the $\delta^{15}\text{N}$ vs $\delta^{18}\text{O}_{\text{NO}_3}$ diagram the principal component slope is in the range of the reported values from the literature, indicating that denitrification is taking place. If this process is controlled by organic matter oxidation we should expect an increase in $\delta^{15}\text{N}$

and $\delta^{18}\text{O}_{\text{NO}_3}$ as the index I_{MO} increases; both relationships are observed. We should also expect a decrease in $\delta^{13}\text{C}$ as I_{MO} index increases, and we observe the opposite relationship, an increase in $\delta^{13}\text{C}$ as I_{MO} index increases. The observed trend can be explained by the dissolution of carbonates, that would increase the HCO_3^- while the $\delta^{13}\text{C}$ values tend to higher values. If the denitrification reaction is taking place via sulphide oxidation we should expect an increase in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ coupled with an increase in I_{p} index, and a decrease in $\delta^{34}\text{S}$ and $\delta^{18}\text{O}_{\text{SO}_4}$ as I_{p} index increases. We observe the two first relationships, but not the latter ones. If SO_4 concentration is fairly constant, these two first relationships could be observed, based on the NO_3^- concentration decrease and the increase in the isotopic composition, even if the denitrification is not linked to pyrite oxidation. In the $\delta^{34}\text{S}$ vs I_{p} index no clear trend is observed, and in the $\delta^{18}\text{O}_{\text{SO}_4}$ the opposite trend is observed.

Garrotxa samples are plotted in Figure 8. In the $\delta^{15}\text{N}$ vs $\delta^{18}\text{O}_{\text{NO}_3}$ diagram the principal component slope is in the range of denitrification if we consider all the samples, but not if we consider the robust estimate. In fact this trend is influenced by 2 samples. Denitrification process probably is only taking place at these two sites. We do not observe a clear trend neither in the $\delta^{15}\text{N}$ vs. I_{MO} index diagram, nor in the $\delta^{18}\text{O}_{\text{NO}_3}$ vs. I_{MO} index one. There is a negative relationship between I_{MO} and $\delta^{13}\text{C}$, though the dispersion of the data is high, as seen by the large size of their variance ellipse. The expected relationships between I_{p} index and sulphate and nitrate isotopes are not observed, in all cases the trend is the opposite. In this area maybe denitrification is weakly occurring at few sites thanks to organic matter oxidation, and SO_4 weathering is an important process which produces the increase in the $\delta^{34}\text{S}$ and $\delta^{18}\text{O}_{\text{SO}_4}$.

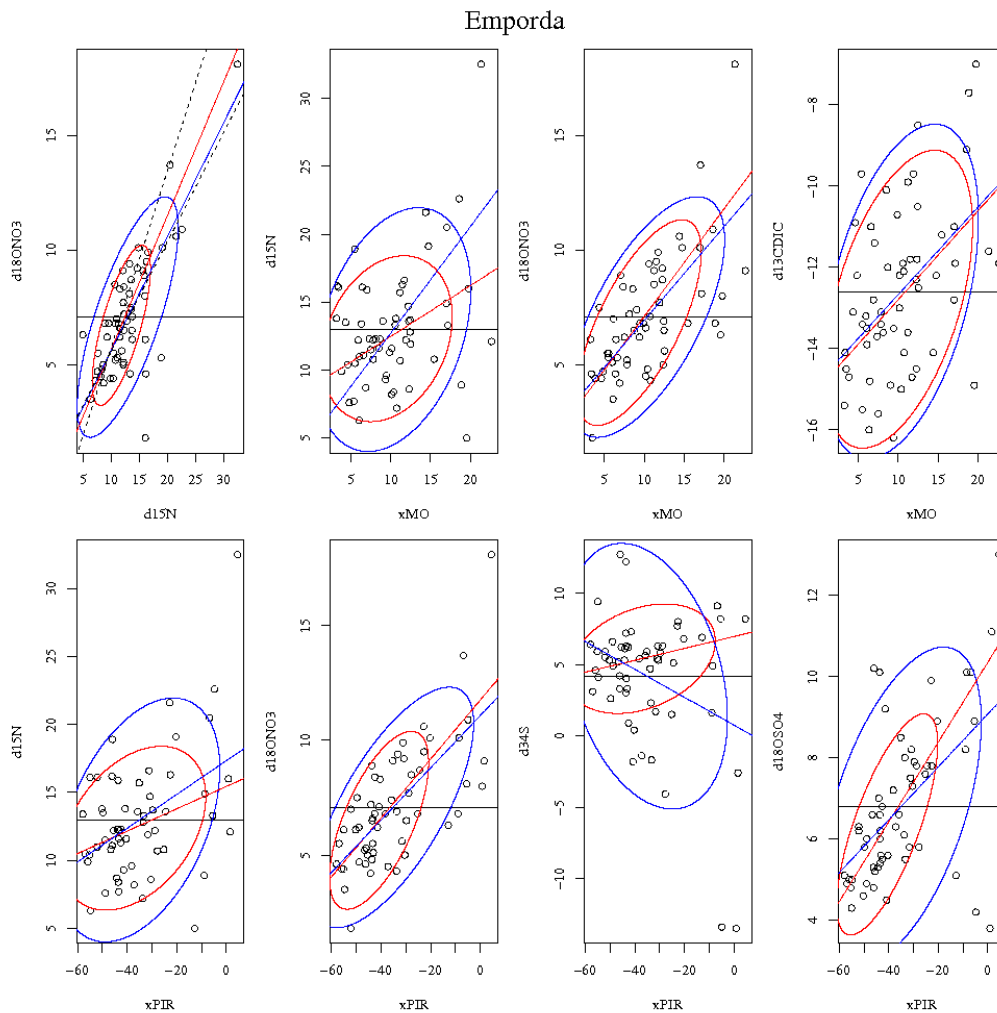


Figure 7: Scatter-plots of those variables related to oxidation processes, for the Empordà area. Ellipses represent radius 2 covariance estimates (red for a minimum-determinant robust estimate, blue for the classical unbiased estimate), and the lines are their first principal component. The first plot shows two dashed lines, with $\epsilon\text{N}/\epsilon\text{O}$ ratios between 1.3 and 2.1, the slope band associated with denitrification

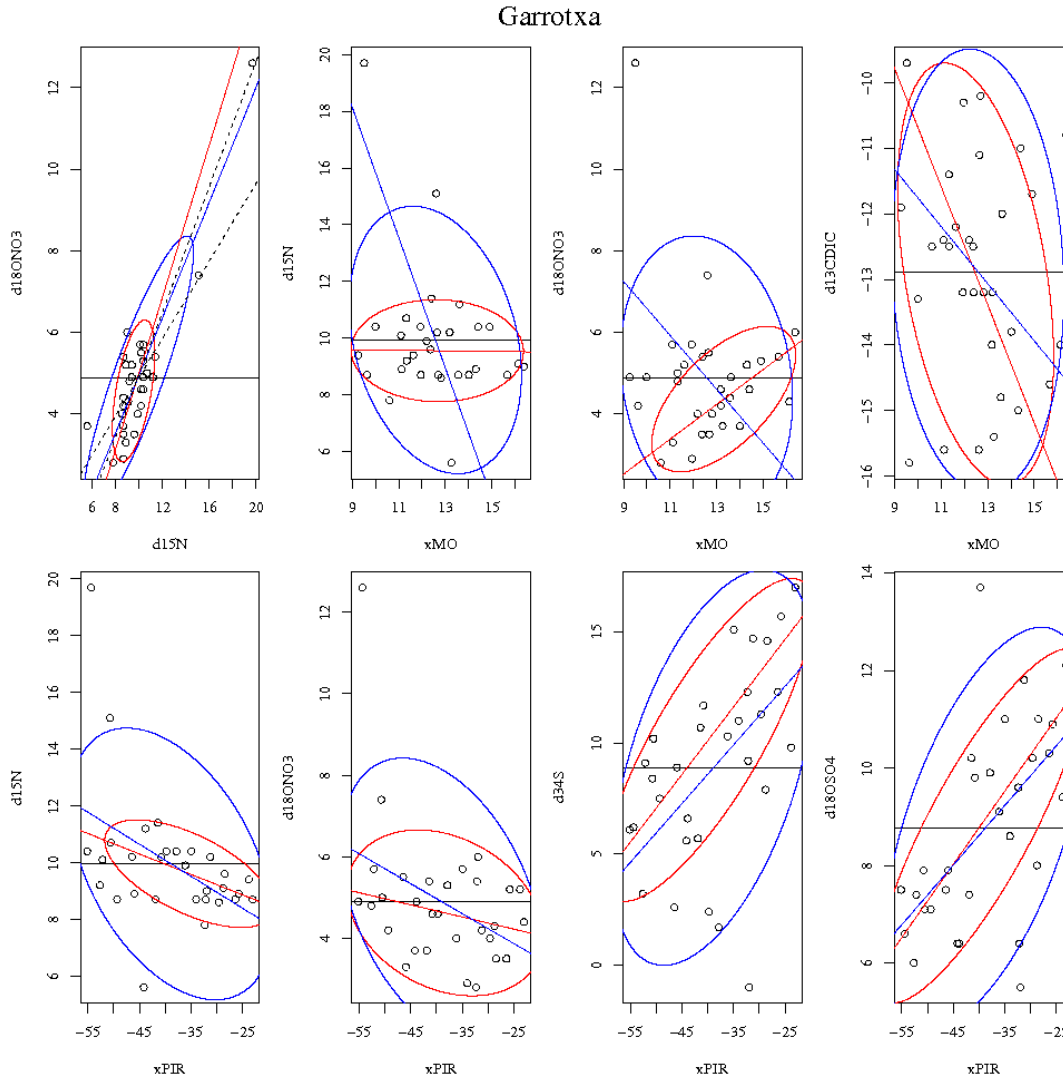


Figure 8: Scatter-plots of those variables related to oxidation processes, for the Garrotxa area. Ellipses represent radius 2 covariance estimates (red for a minimum-determinant robust estimate, blue for the classical unbiased estimate), and the lines are their first principal component. The first plot shows two dashed lines, with ϵ_N/ϵ_O ratios between 1.3 and 2.1, the slope band associated with denitrification

Figure 9 shows the scatter plots of the variables related to oxidation processes in the Maresme area. In the $\delta^{15}\text{N}$ vs. $\delta^{18}\text{O}_{\text{NO}_3}$ diagram the principal component does not follow the expected slope if denitrification is occurring, it has the opposite slope. Denitrification is not taking place in the area. We observe a positive relationship between I_{MO} index and $\delta^{15}\text{N}$. Those samples with higher NO_3^- concentration (lower I_{MO} index) are closer to fertilizers $\delta^{15}\text{N}$ values, which are low. In the $\delta^{18}\text{O}_{\text{NO}_3}$ vs I_{MO} diagram, the robust estimate indicates no relationship, but the classical estimate points to a negative relationship. Those samples with relative higher NO_3^- concentration have higher $\delta^{18}\text{O}_{\text{NO}_3}$ values, and again are closer to fertilizers values. Regarding $\delta^{13}\text{C}$ vs. I_{MO} , a negative relationship is observed, so that higher nitrate concentration is coupled with higher $\delta^{13}\text{C}_{\text{DIC}}$ (this does not agree with the fertilizers $\delta^{13}\text{C}$ values around -25‰). The relationship between I_{P} index and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ suggest also the influence of fertilizers. A positive relationship between I_{P} index and the $\delta^{15}\text{N}$, and a negative relationship between I_{P} and $\delta^{18}\text{O}_{\text{NO}_3}$, indicate that higher nitrate contents, relative to sulphate, have nitrate isotopic values closer to fertilizers values, that is, low values for the $\delta^{15}\text{N}$ and high values for the $\delta^{18}\text{O}_{\text{NO}_3}$. A positive relationship between I_{P} index and $\delta^{34}\text{S}$ and $\delta^{18}\text{O}_{\text{SO}_4}$ is also observed.

According to Vitòria (2004) and Otero et al. (2007), in the Osona area denitrification is taking place by means of pyrite oxidation and the role of organic matter oxidation could not be neither confirmed nor discarded. Figure 10 shows the scatter

plots of the Osona samples. In the $\delta^{15}\text{N}$ vs $\delta^{18}\text{O}_{\text{NO}_3}$ diagram, the principal component follows de denitrification slope, although only the classical estimation, not the robust estimation. Denitrification is taking place in the area. The I_{MO} index shows a positive correlation with the $\delta^{15}\text{N}$, using the classical estimation, not the robust one. The $\delta^{18}\text{O}_{\text{NO}_3}$ also shows a positive correlation with the I_{MO} index, in both estimations. The $\delta^{18}\text{O}_{\text{NO}_3}$ provides a better estimation of denitrification because results are not masked by volatilization processes. This could explain the better relationship observed between I_{MO} index and $\delta^{18}\text{O}_{\text{NO}_3}$. Finally the I_{MO} index shows a negative relationship with the $\delta^{13}\text{C}$ (though only the robust estimation), confirming that denitrification by organic matter oxidation is taking place. A positive relationship between I_{p} index and both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ is also observed. Again the relationship is clearer using the $\delta^{18}\text{O}_{\text{NO}_3}$ because this isotopic ratio is not affected by volatilization processes. The I_{p} index shows a negative relationship with the $\delta^{34}\text{S}$, though the classical and the robust estimations show slightly different slopes. This could be explained by the influence of gypsum weathering in a subset of samples. The I_{p} index does not show the expected negative trend with the $\delta^{18}\text{O}_{\text{SO}_4}$, probably also influenced by the gypsum weathering.

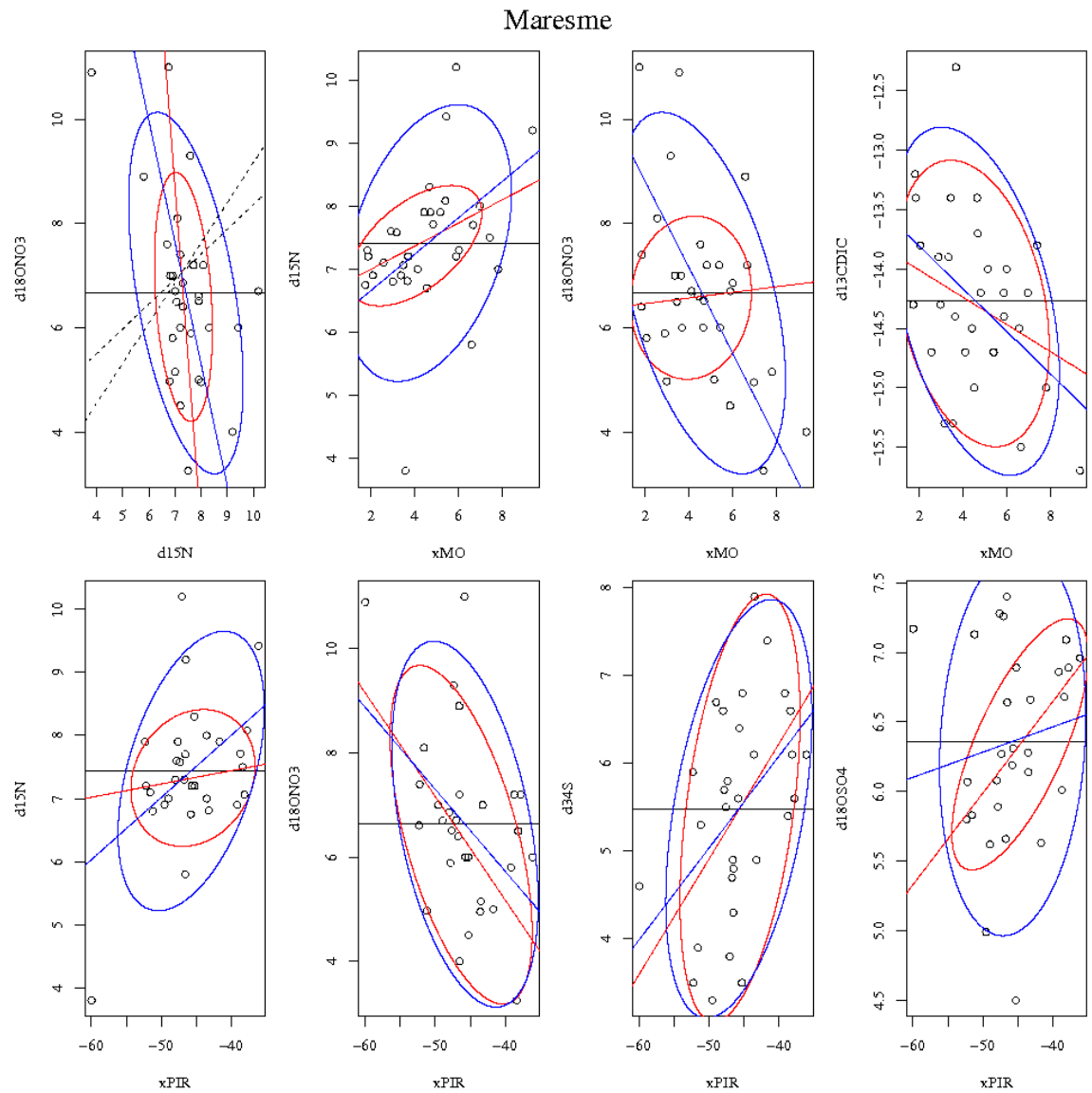


Figure 9: Scatter-plots of those variables related to oxidation processes, for the Maresme area. Ellipses represent radius 2 covariance estimates (red for a minimum-determinant robust estimate, blue for the classical unbiased estimate), and the lines are their first principal component. The first plot shows two dashed lines, with $\epsilon\text{N}/\epsilon\text{O}$ ratios between 1.3 and 2.1, the slope band associated with denitrification

Osona

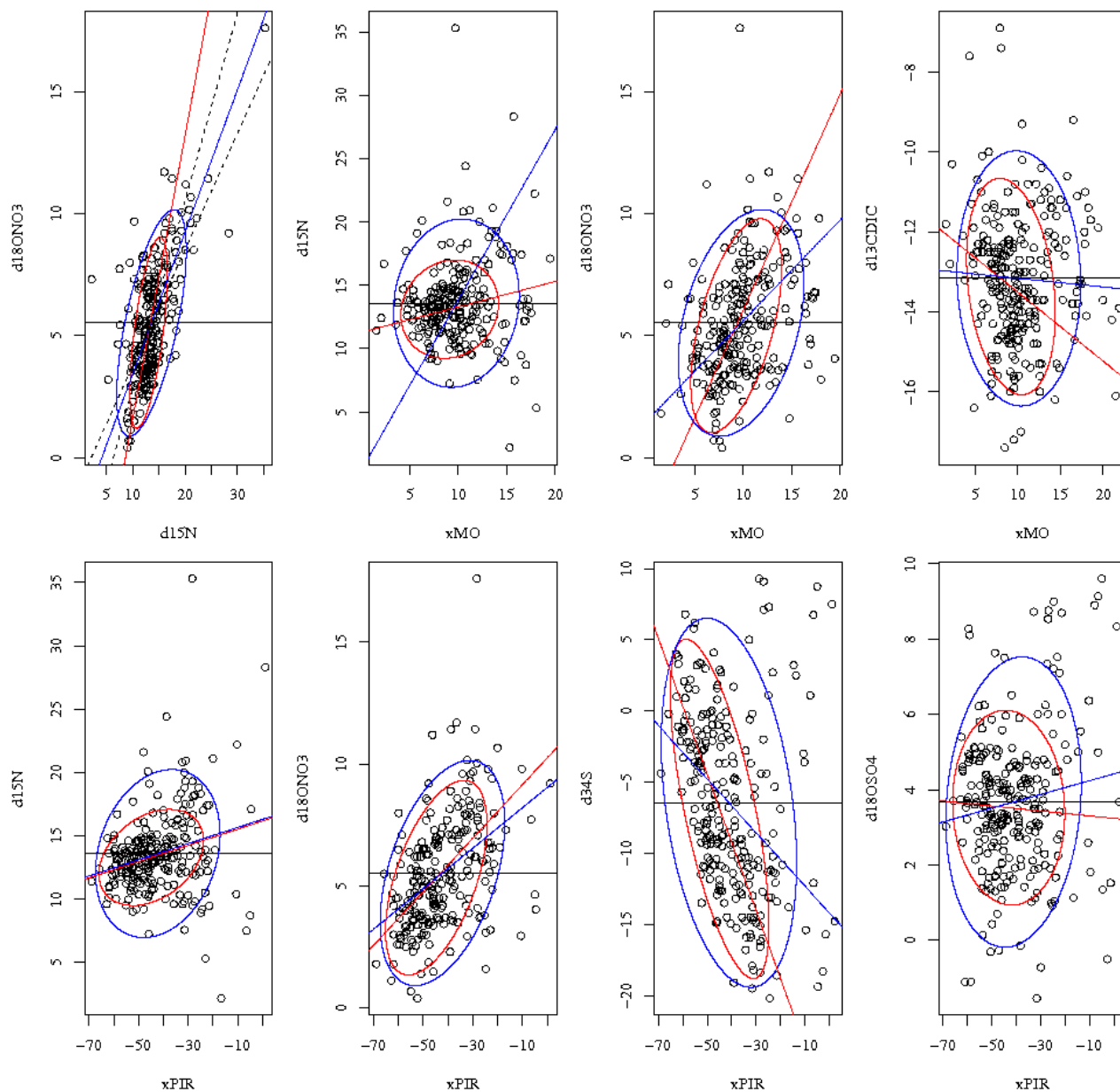


Figure 10: Scatter-plots of those variables related to oxidation processes, for the Osona area. Ellipses represent radius 2 covariance estimates (red for a minimum-determinant robust estimate, blue for the classical unbiased estimate), and the lines are their first principal component. The first plot shows two dashed lines, with ϵ_N/ϵ_O ratios between 1.3 and 2.1, the slope band associated with denitrification

Figure 11 shows the scatter plots of the variables related to oxidation processes in the Selva area. In the $\delta^{15}\text{N}$ vs. $\delta^{18}\text{O}_{\text{NO}_3}$ diagram, the principal component follows the denitrification slope (though only the classical estimation, not the robust one). The I_{MO} index does not show a clear positive trend with the $\delta^{15}\text{N}$, only the classical approach shows a positive slope. The relationship between I_{MO} index and $\delta^{18}\text{O}_{\text{NO}_3}$ does not show a clear trend, and the I_{MO} index vs. $\delta^{13}\text{C}$ shows a positive relationship, the opposite as expected if denitrification by organic matter oxidation is taking place. The observed trend can be explained by the dissolution of carbonates, that would increase the HCO_3^- while the $\delta^{13}\text{C}$ values tend to higher values. In the scatter plot between the I_{P} index and the $\delta^{15}\text{N}$ no clear trend is observed, and though the I_{P} index shows a positive relationship with the $\delta^{18}\text{O}_{\text{NO}_3}$ suggesting the occurrence of denitrification by pyrite oxidation, neither the scatter plot of I_{P}

index with $\delta^{34}\text{S}$ nor the relationship between I_p index and $\delta^{18}\text{O}_{\text{SO}_4}$ confirm this hypothesis. As a conclusion, no clear trend is observed in this area, maybe due to a small sample representation.

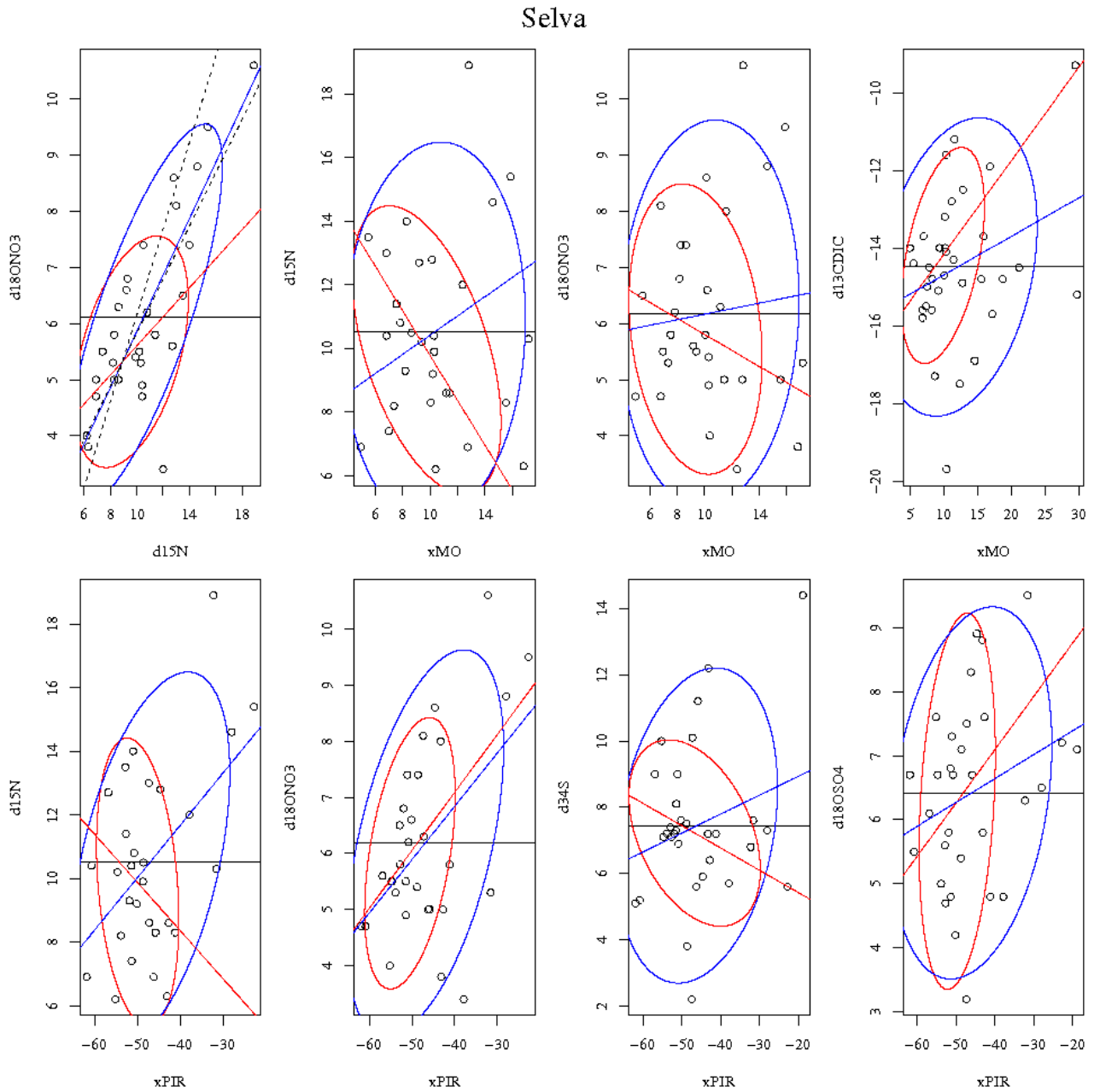


Figure 11: Scatter-plots of those variables related to oxidation processes, for the Selva area. Ellipses represent radius 2 covariance estimates (red for a minimum-determinant robust estimate, blue for the classical unbiased estimate), and the lines are their first principal component. The first plot shows two dashed lines, with $\epsilon\text{N}/\epsilon\text{O}$ ratios between 1.3 and 2.1, the slope band associated with denitrification

As a summary of these relationships, the following table shows the average correlation coefficients of the robust covariance structure (R) between the variables involved in each process (with changed signs for those that should be negative). For each zone, the process with highest average correlation may be taken as the most “reliable” one:

Table 1: Average of the robust correlation coefficients between those pairs of variables linked through each of the two denitrification reactions. Those correlations that *should be* negative has been changed their signs. In this way, the higher this correlation is, the more probable is that process.

	E	G	M	O	S
Organic matter	0.24265	0.11295	0.10696	0.25167	0.22558
Pyrite	-0.08910	0.10190	0.07290	0.31037	-0.03975

Results show that in the Empordà area the most reliable process occurring is organic matter oxidation. In the Garrotxa area denitrification is not clearly taking place, with only two samples with isotopic values in agreement with denitrification processes. In a similar way, in the Maresme area denitrification processes do not occur. In the Osona area both processes are taking place, though the most reliable is pyrite oxidation. In the Selva area, results are not quite clear, but, if denitrification is occurring, the most probable process taking place is organic matter oxidation. Note that the average of the robust correlation coefficients for organic matter denitrification in Selva is approximately as good as in Osona. Interestingly, in the former this process is not sure, and some partial plots do not show a consistent behaviour with it, whereas in the latter we actually know and see (Fig. 10) that it is occurring. Therefore, average-R should be taken with caution: low values are proof that the process is not significant, but high values should be taken as hints, as indicators of its possible presence at a scale general enough in the region. Its actual usefulness is in deciding which of both processes is more reliably active in a given region, once we decided by other means that denitrification actually occurs.

Conclusions

Two useful statistical methods have been applied to the geochemical and isotopic data set of five vulnerable zones, treated as compositional data. The first procedure is the linear discriminant analysis of sample groups, and it allowed assessing the origin of pollution from a different point of view that can confirm the interpretation of isotope data. Secondly, the projection of the geochemical and isotopic variables onto the compositional directions following the two hypothesis reactions (pyrites oxidation, represented by the I_p index, and the organic matter oxidation, by the I_{MO} index) means a good approach to determine the role of organic matter oxidation in denitrification processes occurring in the areas. Up to now, with a classical approach this role could not be neither confirmed nor discarded, and $\delta^{13}C$ -DIC values could not give clear information. Therefore, these compositional analysis applied to our data can become a further step in the evaluation of denitrification processes.

Acknowledgements

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