

# Isotope Ratio - Mass Spectrometry

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**Abstract.** This article summarizes the configurations involving isotope ratio mass spectrometry (IRMS) technology available at the CCiTUB and the wide range of possible applications. Some examples of these applications are shown.

## 1. Introduction

The natural abundances of stable isotopes for any given element are not constant in nature due to a great variety of physical and chemical fractionation processes that constantly occur. The isotope ratio mass spectrometry (IRMS) is a technology that allows us to measure these small variations in isotopic abundances of the major light elements in nature (H, C, N, O, S) with high precision.

## 2. Methodology

An isotope ratio mass spectrometer consists of three main parts:

- Electronic ionization source (high stability and yield of ionization)
- Magnetic field analyzer (effective separation of different ion beams)
- Multiple Faraday cups detector (robust collection of the specific ions)

Gas sample introduction can be performed directly by means of dual inlet (DI) systems or via an additional hyphenated technique, referred to as continuous flow (CF) systems, through a carrier gas, e.g. chromatography, elemental analysis, pyrolysis. Both systems are available at the Scientific and Technological Centers of the University of Barcelona, comprising several isotope ratio mass spectrometers with different sample preparation devices. These peripherals are carbonate device, equilibration-GC system (gas bench, GB), elemental analyzers (EA), pyrolyzers (TC/EA), gas chromatographs (GC) and liquid chromatograph (LC).

Such variety of configurations allows a full range of applications in different areas:

- Life and Earth sciences (physiology, ecology, hydrology, climate, marine geosciences, biogeochemistry)
- Health sciences (biochemistry, metabolism, physiology, nutrition)
- Forensic science
- Archaeology

The different applications comprise the analysis of samples of natural isotopic composition and low level of enrichment (up to 2-3% heavy isotope), where isotopes can be used as tracers of metabolic and fluxomic pathways.

In our laboratories we have implemented several routine measurements for specific type of samples, covering inorganic and organic fields. The stable isotope analyses that we perform in our laboratories are shown in Table 1.

## 3. Examples of applications

### 3.1. DI-IRMS. Carbonate analysis in foraminifera.

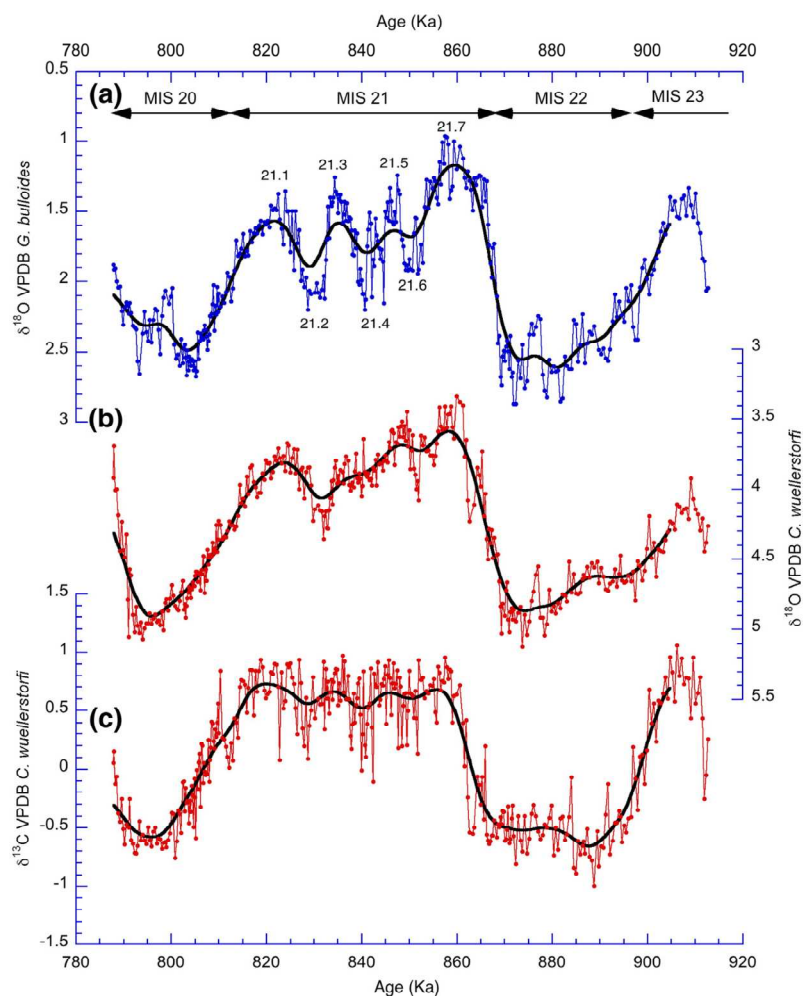
Foraminifera have been distributed widely in the ocean from the Cambrian period to the present. Their hard shells, mainly consisting in  $\text{CaCO}_3$ , are often preserved within seafloor sediment as microfossils. The analysis of their stable carbon and oxygen isotopic compositions ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) is useful because these parameters are paleoindicators of the paleoenvironment at the time when they were alive.

To analyse the stable isotopic composition of foraminiferal shells, more than 20 micrograms of  $\text{CaCO}_3$  are needed. In order to obtain a reliable result, the weight of samples and reference materials should be similar. This is why samples are weighted prior to be transferred to a reaction tube, which is placed in the automatic carbonate device feed system. The carbonate device works according to the McCrea method (McCrea, 1950). This method releases  $\text{CO}_2$  from the  $\text{CaCO}_3$  and the gas flows into the IRMS (see Fig. 1).

**Table 1.** Stable isotope analyses performed at the CCiTUB by using different IRMS configurations

Technique	$\delta^{13}\text{C}$	$\delta\text{D}$	$\delta^{15}\text{N}$	$\delta^{18}\text{O}$	$\delta^{34}\text{S}$
<b>DI-IRMS</b>	Carbonate microsamples			Carbonate microsamples	
<b>GB-IRMS</b>	$\text{CO}_2$ from air	Water samples		Water samples	
	DIC in water samples			$\text{CO}_2$ from air	
<b>EA-IRMS</b> <b>TC/EA-IRMS</b>	Solid and liquid bulk material	Solid and liquid bulk material	Solid and liquid bulk material	Solid and liquid bulk material	Solid and liquid bulk material
<b>GC-IRMS</b>	Specific Organic compounds (VOCs and SVOCs)	Specific Organic compounds (VOCs and SVOCs)	Nitrogenated organic compounds		
<b>LC-IRMS</b>	Water soluble organic compounds (total extract and compound specific)				

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**Figure 1.** Example of isotope records in an oceanic core (Ferretti et al, 2010).

### 3.2. CF-IRMS

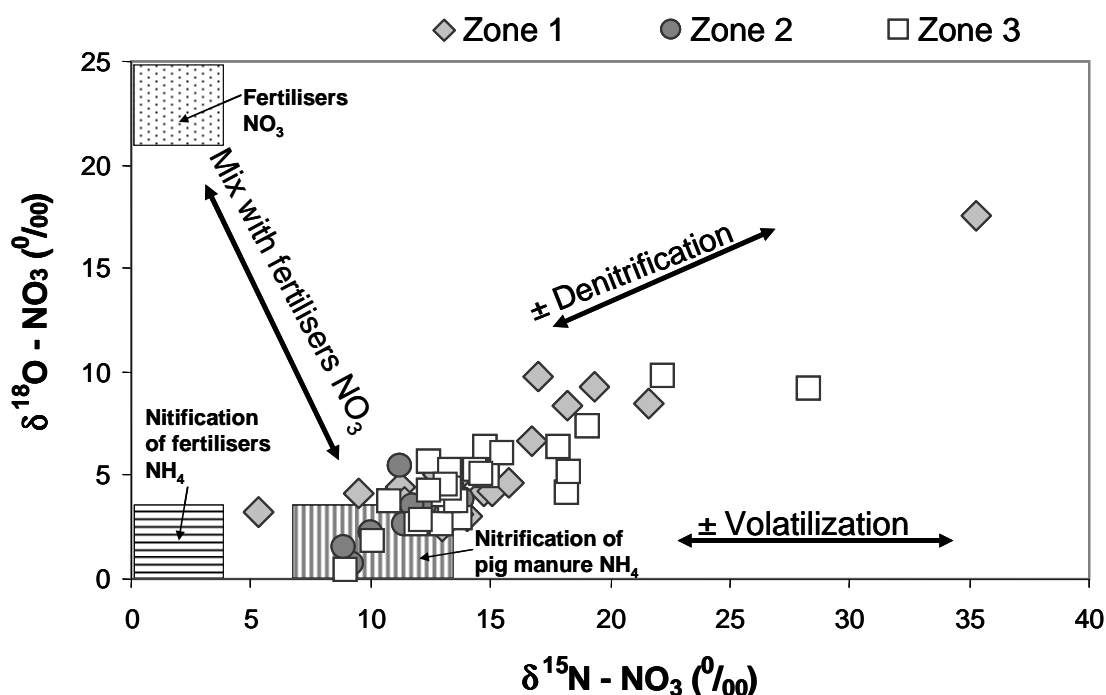
At our IRMS facilities, several methodologies have been developed in CF-IRMS. Some of them are focused to environmental applications as the stable isotopes signatures contribute to the knowledge of the origin and fate of specific pollutants found in different areas. Moreover, the isotopic analyses contribute to control the natural or induced degradation processes (remediation). Other applications are related to metabolism and physiology, where stable isotopes are used as tracers of different processes.

#### 3.2.1. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of dissolved nitrates in waters .

The analysis of the isotopic composition of the ions involved in the denitrification reactions allow us to determine which processes control the natural attenuation (Otero et al., 2007).

In this example sample preparation is as follows. For the  $\delta^{15}\text{N}_{\text{NO}_3}$  and  $\delta^{18}\text{O}_{\text{NO}_3}$  analysis, dissolved nitrates are concentrated using anion-exchange columns, after extracting the sulphates and phosphates by precipitation. Afterwards, dissolved nitrates are eluted with HCl and converted to  $\text{AgNO}_3$  by adding silver oxide. The liophilised  $\text{AgNO}_3$  is enclosed in a Sn and/or Ag capsule for the subsequent  $\delta^{15}\text{N}$  and/or  $\delta^{18}\text{O}$  analysis by EA-IRMS and/or EA/TC-IRMS.

The isotopic values  $\delta^{15}\text{N}$  vs.  $\delta^{18}\text{O}$  of dissolved  $\text{NO}_3^-$  found in different sampling sites of a regional area near Barcelona (Spain) are shown in Fig.2. The isotopic composition of nitrates indicates that dissolved nitrate is mainly derived from pig manure ammonium which is nitrified at the aerobic zone. It is worth noting that the  $\delta^{18}\text{O}_{\text{NO}_3}$  indicates that nitrate is derived from fertiliser  $\text{NH}_4$ , which has been nitrified. There is no evidence of the contribution of “direct” nitrate from fertilisers. Samples show a coupled increase in  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}_{\text{NO}_3}$ , with a slope 2:1. This correlation indicates the existence of denitrification processes, in almost one third of the samples.

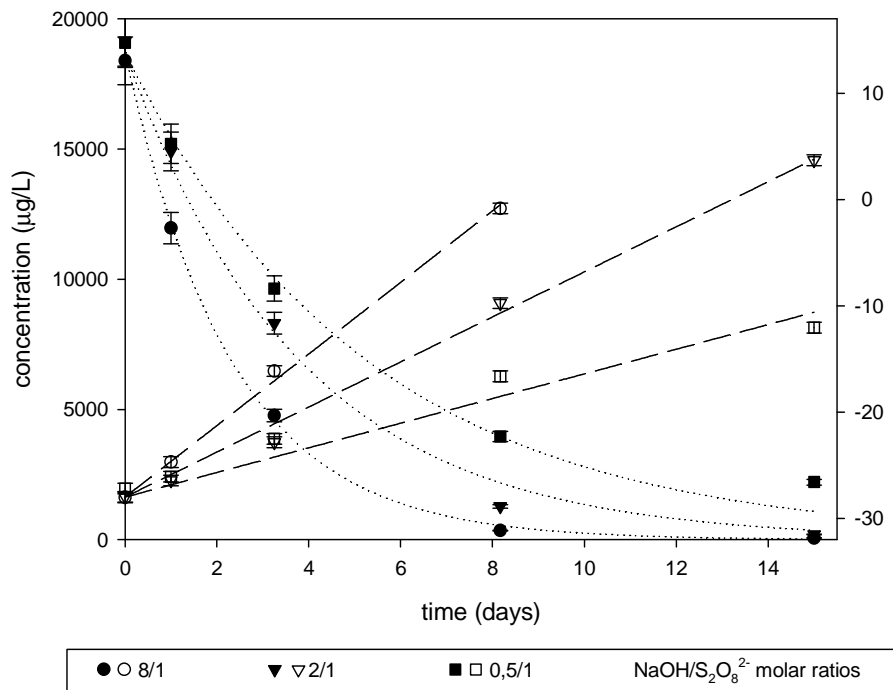


**Figure 2.** Isotopic values  $\delta^{15}\text{N}$  vs.  $\delta^{18}\text{O}$  of dissolved  $\text{NO}_3^-$  found in different sampling sites of a regional area near Barcelona (Spain). . Arrows indicate the isotopic changes linked to the different processes.

#### 3.2.2. Compound specific carbon isotope analysis of volatile organic compounds

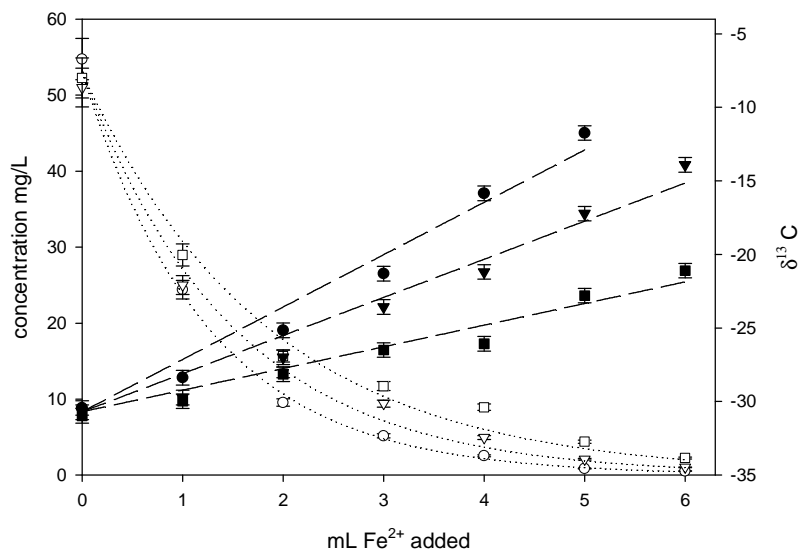
Using head space-solid phase microextraction (CAR-PDMS fiber) as a preconcentration technique, it is possible to detect trace levels (LOQ <10 ug/l) of halogenated and non-halogenated volatile compounds and analyze its isotopic composition with optimum precision (J.Palau et al., 2007)

As in-situ chemical oxidation has emerged as an effective and low-cost method for contaminated groundwater remediation, analysis of the variation of the isotopic composition of the halogenated contaminants during different oxidation conditions was performed in order to evaluate them as a potential tool to monitor the performance of the oxidative processes applied. The observed trend in the isotopic values of  $\delta^{13}\text{C}$  allowed the quantification of the enrichment factor using Rayleigh equation (see Figs. 3 and 4).



**Figure 3.** Concentration (black symbols) and  $\delta^{13}\text{C}$  values (white symbols) of TCA for different  $\text{NaOH}/\text{S}_2\text{O}_8^{2-}$  molar ratios (Marchesi *et al.*, 2009).

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**Figure 4.** Concentration (white symbols) and  $d13\text{C}$  values (black symbols) of PCE for different  $\text{S}_2\text{O}_8^{2-}/\text{Fe}^{2+}/\text{PCE}$  molar ratios (Marchesi *et al.*, 2009)

3.2.3. Study of <sup>13</sup>C and <sup>15</sup>N isotopic labeling on amino acids in plants by GC/C/IRMS

We have developed a methodology that allows the detection and quantification of both unlabelled and isotopically labeled amino acids so that it can be applied in a broad range of metabolomic and fluxomic studies in which stable isotopes can be used as tracers (G. Molero et al., 2011). Typical results are shown in Figs. 5 and 6.

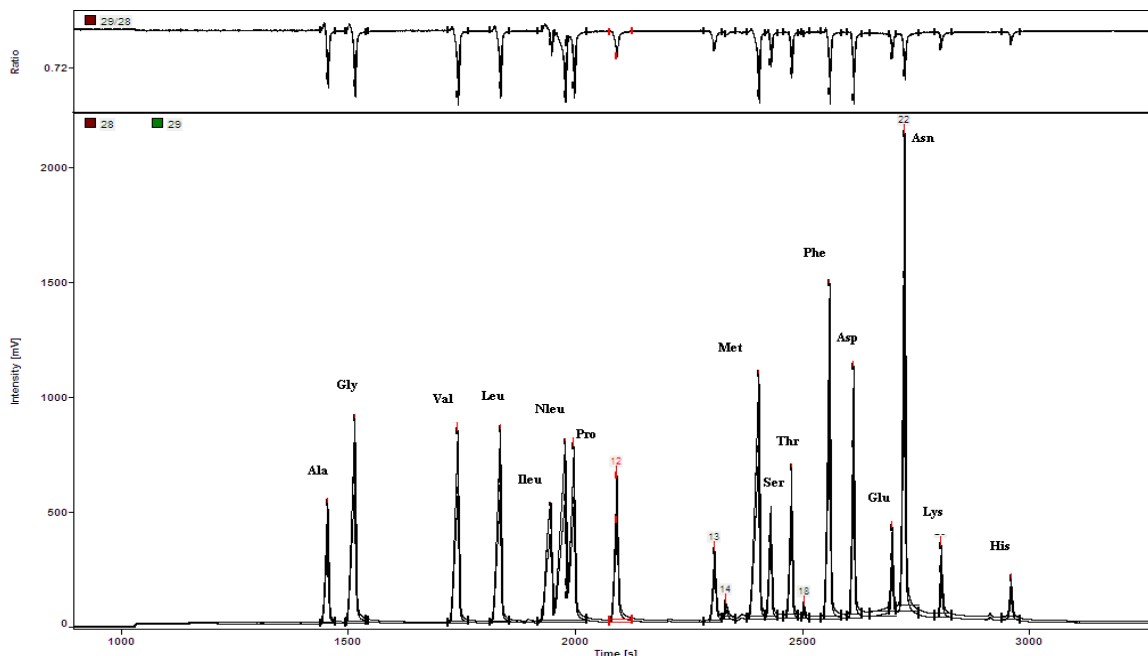


Figure 5. GC/C/IRMS chromatogram obtained from a mixture of aminoacids (MTBDMS-derivatives) on a PT-5 column

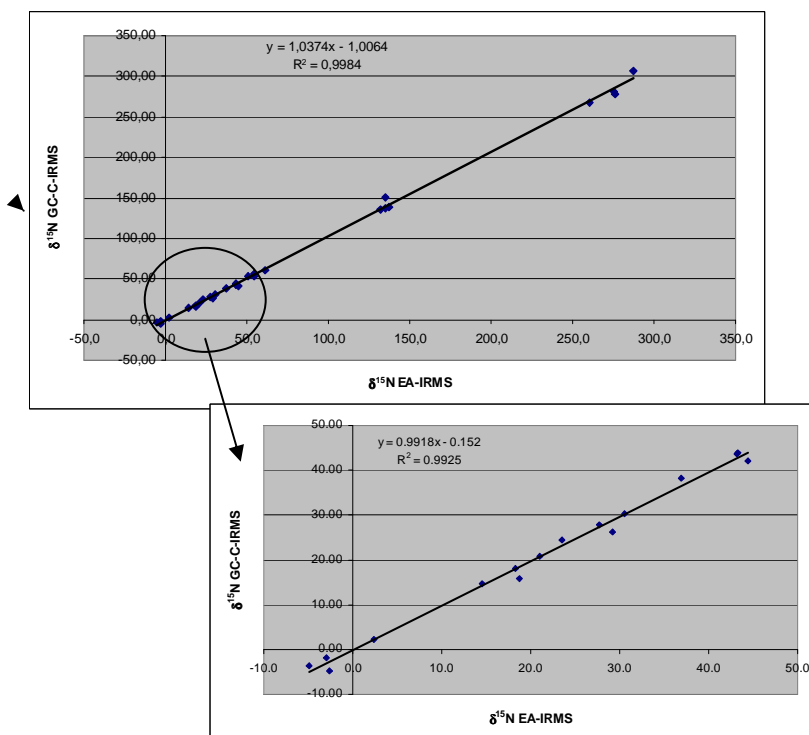


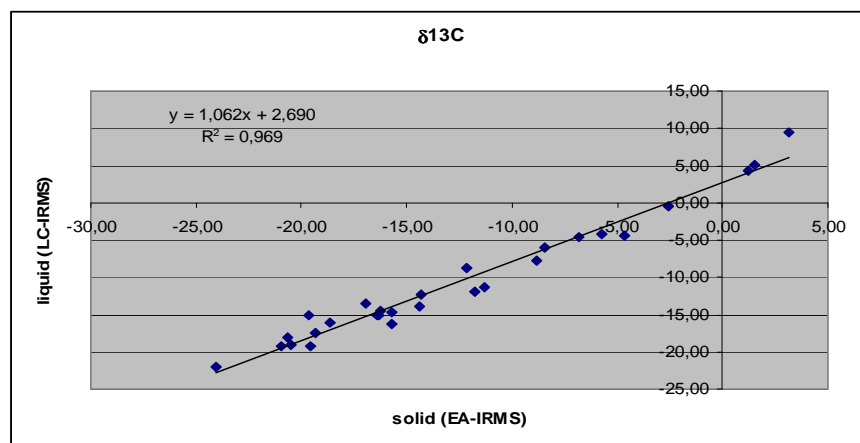
Figure 7. Comparison between  $\delta^{15}\text{N}$  values obtained by EA-IRMS and GC-C-IRMS

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A good linearity correlation ( $r^2 > 0,99$ ) was observed between  $\delta^{15}\text{N}$  signature values ( $n = 28$ ) obtained by EA-IRMS vs. GC-C-IRMS of four amino acids (Ala, Phe, Asp, Glu), ranging from natural to enriched (up to 300 per mil) isotopic composition. As a result, the developed methodology is applicable for natural abundance as well as for enriched materials.

### 3.2.4. Analysis of $\delta^{13}\text{C}$ in water soluble compounds by FIA-IRMS and LC-IRMS

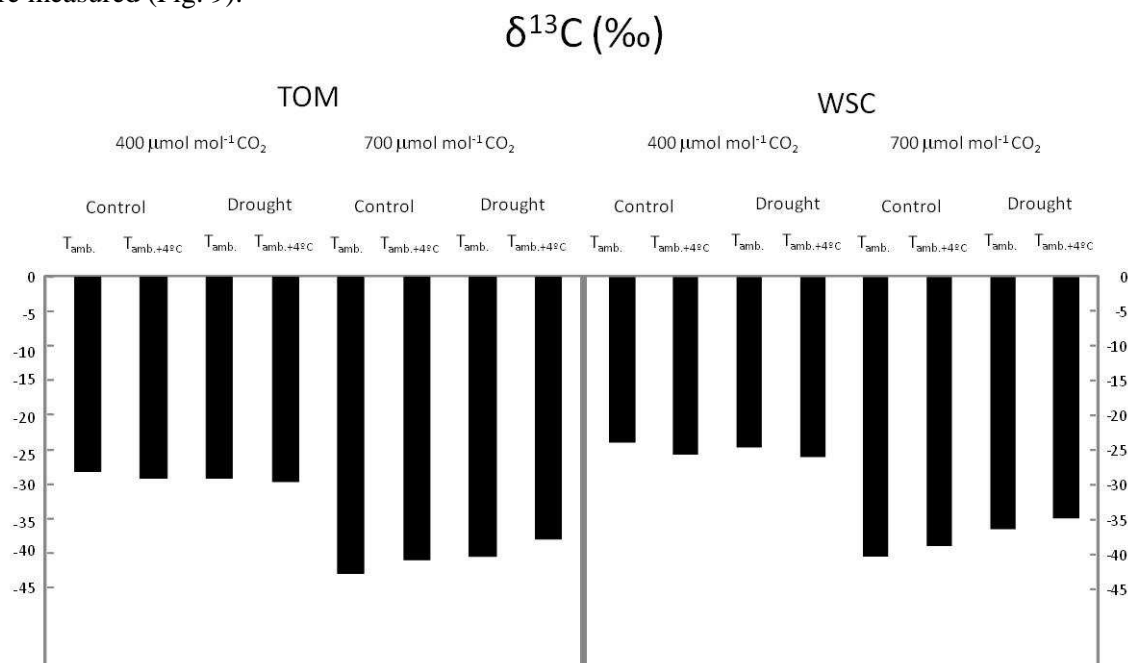
The flow injection analysis FIA-IRMS technology allows us to analyse fast the bulk carbon isotopic composition of very small water soluble samples (50ul, 100ngC) without the complex sample preparation required for EA-IRMS analysis (see Figs. 8).



**Figure 8.** Comparison between  $\delta^{13}\text{C}$  values (water soluble compounds from plants) obtained by EA-IRMS and FIA-IRMS

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The methodology has been applied to assess the performance of plants exposed to contrasted conditions of depleted  $\text{CO}_2$ , temperature and water availability (.Aranjuelo, 2011). The technology is useful as a fast screening method for metabolic response as the recently assimilated compounds are measured (Fig. 9).



**Figure 9.** The  $\delta^{13}\text{C}$  values obtained from WSC are less  $^{13}\text{C}$  depleted in comparison to those obtained from total organic matter by EA-IRMS, which reflect better the stressful conditions applied.

## References

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