ON-LINE MEASUREMENT OF $^{13}C/^{12}C$ AND $^{15}N/^{14}N$ RATIOS BY E/A-DILUTER-IRMS

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Abstract. Efficient food control requires rapid procedures for testing source authenticity. Food is produced inside a closed 'isotopic environment' from where it inherits a specific isotopic composition or fingerprint. Isotope ratio mass spectrometry (IRMS) measures isotopic compositions using simple gases like CO_2 or N_2 exclusively. From food samples these gases may be produced by combustion in a commercial CHN analyser (Elemental Analyser, EA). Following GC separation of the combustion gases the elemental content is determined using a thermal conductivity detector (TCD) . The effluent of the EA is coupled to the mass spectrometer via an open split. Because the relative amounts of the bio-elements vary significantly, (often C/N is 25/1 or larger), the amount of analyte gas produced from a single sample must be adjusted e.g. using a diluter. Our diluter configuration can be adjusted to measure repeatedly the $^{13}C/^{12}C$ ratio of carbon dioxide in mineral waters, as well as to measure $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios from biological or soil samples simultaneously. In the first application different types of carbon dioxide, produced naturally (well) or technically (process), can be distinguished. The second application offers the possibility to trace the fate of a fertilizer in vineyards by determining the isotopic variation of nitrogen and carbon in soil and vines.

1. INTRODUCTION

Food control using natural stable isotope variation is the most modern tool for checking authenticity. In contrast to 'quality' (chemically pure), 'authenticity' refers to the correct origin and natural composition. A particular food may be without risk for consumers, but still may not originate from the region of the production proposed or be of exclusively natural composition. At first glance this may not constitute a disadvantage for consumers. However, it changes competition on the market and it certainly is not favourable for the consumer or the environment. Food production in Europe is the most important factor determining use and hence structure of the landscape. While the European Union spends billions of EC to support the original pattern of local agricultural production, more money is lost by illegal production of food inside and outside the EU.

Modern traffic enables a quick translocation of materials. Open borders and different administrative systems render backtracing of material by registration obsolete. Therefore, the fingerprint of the natural isotopic composition of a product probably is the only tool to reconstruct its individual history. Produced or formed in its local 'isotopic environment' which varies with the individual geographical location food gets a non-changable isotopic fingerprint. Usually control authorities require and enforce a rapid analysis of a product. For such rapid screening the overall isotopic data are sufficient in many cases.

The paper presents two examples of such a screening procedure used in daily routine. First, the $^{13}C/^{12}C$ ratio of carbon dioxide in mineral water is measured to distinguish between gas from wells and from technical processes. German regulations for the declaration of commercially distributed mineral waters require this differentiation. The second application is a study of the transfer of nitrogen from fertilizer to wine through the soil using $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios. In a vineyard top soil vegetation and wine are competing for nutrients. Both isotope pairs are measured in sequence within one run.

2. METHOD

2.1. On-line measurement technique

Our general method for stable isotope analysis is the on-line or continuous-flow technique in isotope ratio mass spectrometry IRMS [1]. The material is converted to chemically simple gases by burning

and reducing it in a set of two furnaces inside an elemental analyser (EA). The gases produced are then separated gas chromatographically. The sample effluent outlet is fed into an isotope ratio mass spectrometer via an open split by a small capillary inlet. The diameter and length of this capillary determine the split and thus the amount of gas introduced into the ion source of the mass spectrometer. The arrangement carefully avoids isotopic fractionations which may occur during diffusion processes. No alteration of the flow during measurement is allowed.

As a consequence a different means of varying the sample amount flowing into the mass spectrometer is needed, in our case a diluter modul . This device can divide and thereby dilute the gas flow by connecting the parallel flow lines of carrier gas. One line flushes through the elemental analyser-TCD-GC branch (sample-helium-flow), the other carries the reference gas. Both lines are linked by a capillary (Fused Silica, 10 cm, 150 µm). For samples containing larger amounts of gas, e.g. carbon dioxide in mineral waters, the diluter may be used to reduce the concentration of the sample gas in the carrier stream. An enhanced pressure of carrier gas is employed to transport the gas sample through the tubes. In front of the open split the concentration of the sample gas is reduced by the diluter. In a similar manner, measurements of biological material, e.g. when measuring $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios simultaneously, require the dilution of at least one of the compounds during a single run, mostly that of carbon dioxide.

All isotopic ratios are related to IAEA standards in theory. In practice they are linked to the certified gases *Isotop*® supplied by Messer-Griesheim Krefeld. Each gas sample has its own certificate according to the regulations of the ISO 9000 series. The notations are given as δ-values related to the IAEA standards.

2.2. On-line measurement of carbon dioxide in mineral waters

Fig. 1 schematically shows the arrangement of the head space extraction from a liquid sample together with the continuous-flow EA-IRMS-device. A gas sample for instance from a closed bottle of mineral water can be fed into the continuous flow system of the commercial equipment. The diluter is the central unit of the extraction equipment represented by a programmable valve-switch which can be handled by the IRMS software (VG Isochrom Software 1.67). In general the diluter acts as a „flow divider" aimed at adjusting the carbon dioxide to the dynamic range of the MS. This is necessary, because nitrogen in contrast to carbon is a minor component in most biological substances (except urea). For diluting and hence adjusting CO_2 the reference helium flow of the element analyser is used. It is directly coupled to the sample helium flow with a capillary so that approximately 10% of the sample helium flow is obtained. While an open diluter valve directs the flow to the main carrier, the closed valve connects the reference helium flow to the MS. For our application the diluter is used in a different way. Its function is to generate carbon dioxide peaks from a continuous carbon dioxide flow. Consequently, the reference gas line is connected to the extraction facility. For carbon dioxide analysis in mineral waters, the pressure in the extraction equipment (V1, V2, V5 open, V3, V4 closed) was allowed to drop to 5 mbar. After piercing the mineral water bottle by a needle, the carbon dioxide flows through the water trap to the gas reservoir GH (V1, V3, V4 closed, V2, V5 open).

With helium added to the gas reservoir the pressure is allowed to rise to 1.1 bar (Valves V1, V2, V4, and V5 closed, V3 open). The pressure enables a continuous transfer of carbon dioxide into the helium carrier (V4 and V5 open). A fused silica capillary (length 150 mm) is used as a flow resistance between the extraction equipment and the helium line. The length of the capillary, the amount of carbon dioxide taken out of the mineral water bottle and the pressure in the gas reservoir regulate the amount of carbon dioxide in the carrier. The technical arrangement enables consecutive measurements from the same sample by switching the valve gear of the diluter (switching time: ~ 2 sec.). A typical run is shown in Fig. 2.

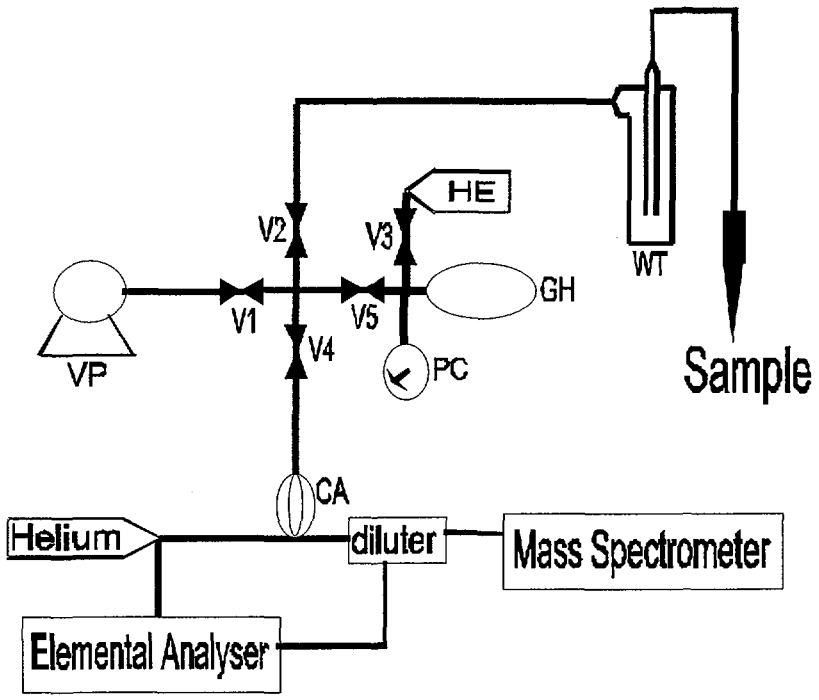

Fig. 1. Schematic representation of extraction equipment (WT – water trap [–90°C]; GH-gas reservoir[250ml]; VP – vac. pump; CA – capillary; V1..5 – valves; HE – helium; PC – pressure gauge.

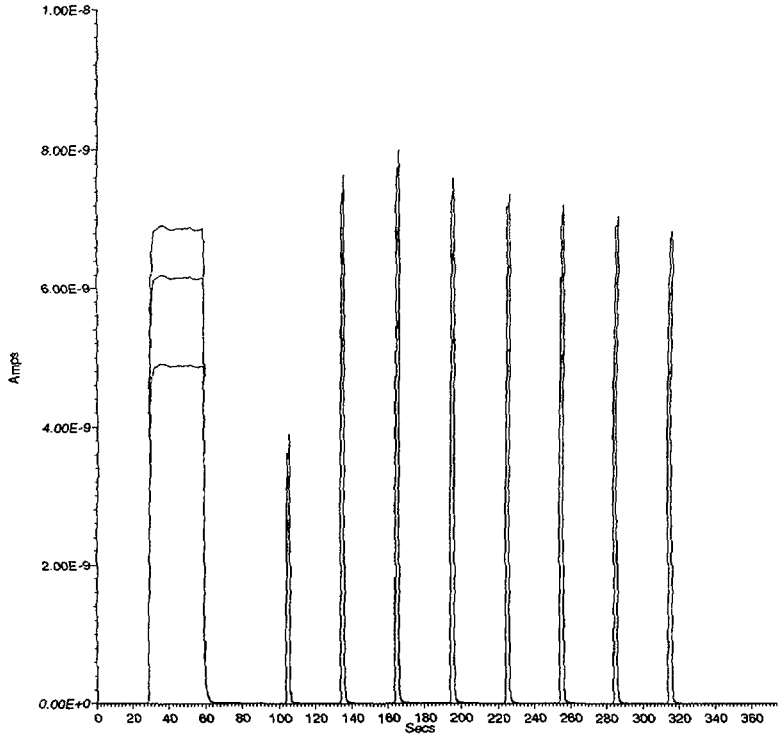

Fig. 2. Plot of a $^{13}C/^{12}C$ run of carbon dioxide extracted from mineral water. The first peak is generated by the reference gas, the subsequent spikes are separate samples gas injections from the gas reservoir (see text).

2.3. Simultaneous measurement of two isotope ratios

The initial intention of the diluter application was to enable a simultaneous determination of more than one isotopic ratio within a single run. For the determination of the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratio of biological material an example is given. The simultaneous measurement of both isotope ratios is possible only when at least one gas is diluted to a suitable range of detection. Systematic effects, in particular isotopic fractionation within the flow system, have to be taken into account.

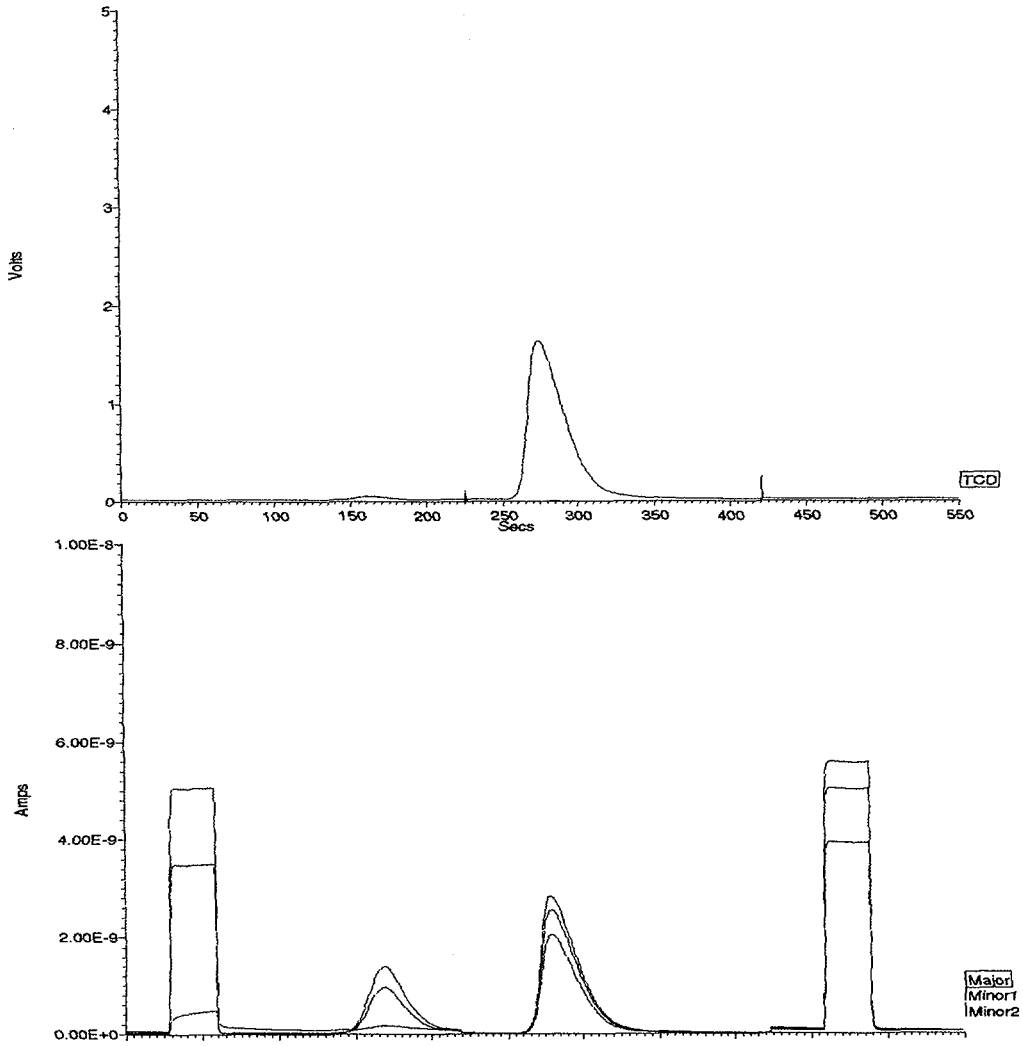

Fig. 3. Run of atropin standard (upper part: TCD signal; lower part: mass spectrometric record of the gases exhausting from the EA., Nitrogen is eluting first, followed by the carbon dioxide) The chromatogram is bracketed by gas injections of N_2 and CO_2 reference gas respectively. Switching of the high voltage and hence the mass position occurs at 225 seconds.

After precise weighing with a Sartorius microbalance samples are wrapped into tin foils . The weight data are fed into the data aquisition system EA 1.67 (Micromass). From an autosampler the samples drop into the hot zone of the EA where they are combusted at 1021° in a pure temporary oxygen environment (purity level 5.8). From here the combustion product gases are swept with helium (purity 6.0) through a reduction furnace filled with copper at 650° . Traces of water are trapped on magnesium perchlorate. The oxidation furnace was packed with tungsten-VI oxide, silver wool and cobalt-II/III oxide coated by silver. The whole packing is kept in place by quartz wool. The filling is cleaned

(removing the ash) or changed after about 600 samples. The copper in the reduction reactor usually is oxidized after 200 samples and has to be reduced by fushing with hydrogen under heating. Each series of samples is controlled by regularly including measurements of a standard substance of known elemental as well as isotopic composition. Following combustion, reduction and water removal the gases are separated gaschromatographically (Porapak Q, 2 m). The concentration of the compounds is determined by a TCD (termal conductivity detector). The retention times under the conditions of operation are 160 s for nitrogen and 275 s for carbon dioxide. A typical run is shown in Fig. 3. The TCD signals of nitrogen and carbon dioxide exhibit a ratio of about 1 : 25, which is typical for plant material. In the case shown the reference substance used is atropin. Table I demonstrates a typical example from a whole sequence run to illustrate the precision of the technique. The dummy samples preceeding each run are not shown.

TABLE I. PART OF A TYPICAL RUN OF ATROPIN STANDARDS AND TWO IAEA-NITROGEN STANDARDS (RAW DATA).

Sample	δ ($^{13}C/^{12}C$) ‰ v.s. PDB	stand. deviation $^{13}C/^{12}C$	δ ($^{15}N/^{14}N$) ‰ v.s. air-N_2	stand. deviation $^{15}N/^{14}N$
Atropin	-3.9		-17.8	
Atropin	-3.9		-17.9	
Atropin	-4.1		-17.4	
Atropin	-3.8		-17.5	
Atropin	-3.9		-17.3	
Atropin	-4.0		-17.5	
Atropin	-3.8		-17.8	
Atropin	-3.9	0.1	-17.5	0.2
IAEA-N1			2.6	
IAEA-N1			2.5	
IAEA-N1			2.6	
IAEA-N1			2.4	0.1
IAEA-N2			22.6	
IAEA-N2			22.2	
IAEA-N2			22.4	
IAEA-N2			22.4	0.2

3. RESULTS

3.1. $^{13}C/^{12}C$ of carbon dioxide in mineral water

Carbon dioxide of commercial mineral waters can vary significantly (Fig. 4). There are two main sources of carbon dioxide as revealed by their different $^{13}C/^{12}C$ ratios. The $^{13}C/^{12}C$ ratios of carbon dioxide from wells range between –4 to –8‰ vs. PDB ('well carbon dioxide'). The wells are mainly supplied with CO_2 by recent volcanic activity in the Eifel and Black Forest region. The second group of data between –26 and –44‰ represents carbon dioxide mainly manufactured by burning coal or biomass ('processed carbon dioxide'). This carbon source is dominated by plants using the C_3 photosynthesis pathway, the most common type in plants of our area. German mineral water regulations allow a specific qualification of the mineral water ('carbon dioxide of its own well') to be used on the label only when the carbon dioxide originates exclusively from its own well. Usually customers honor this by paying a higher price. The notion behind the declaration suggests that the mineral water in question is a pure natural product. Processed carbon dioxide may not differ in terms of quality but the mineral water is not received as an entirely natural product. The regulation is unique in Europe.

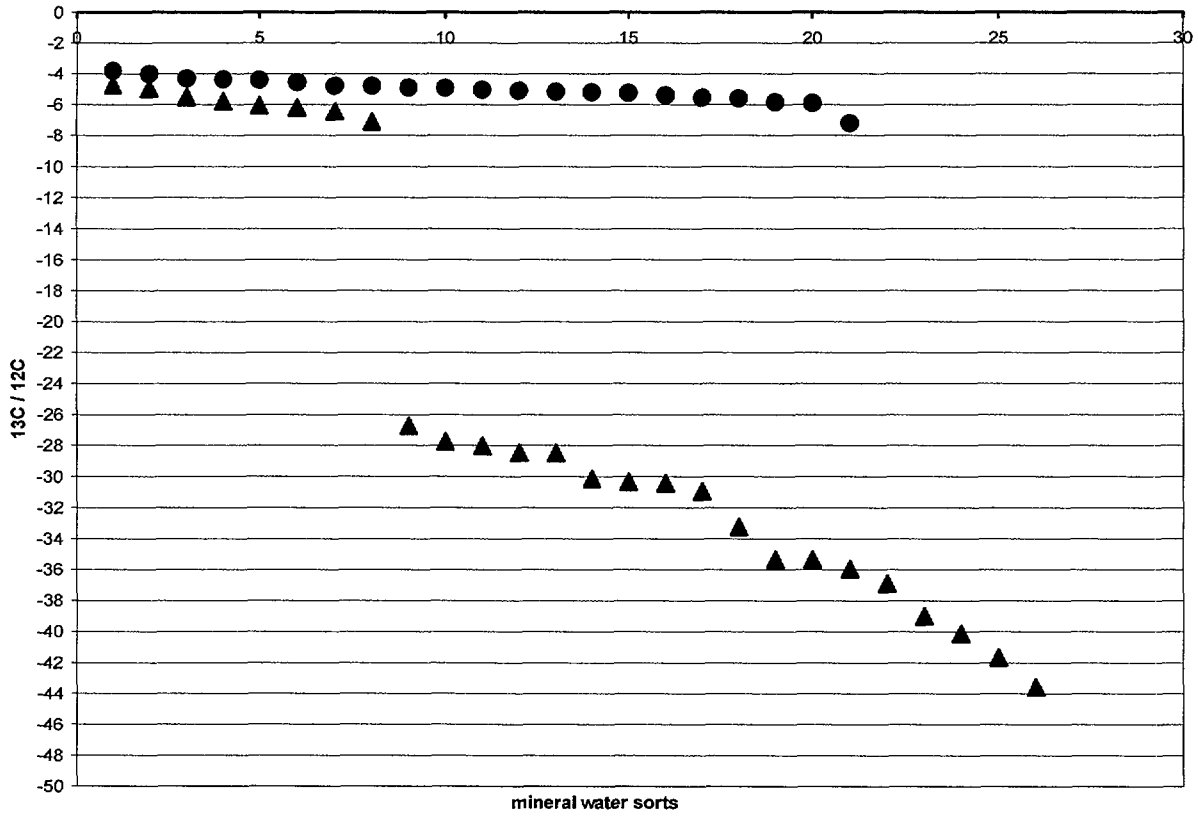

Fig. 4. $^{13}C/^{12}C$ ratio of carbon dioxide in mineral waters (dots: declared as carbon dioxide bottled at its own well; triangle: no declaration, hence technical carbon dioxide and carbon dioxide originating from another well).

Consequently suppliers of mineral water are not allowed to declare their product as carbonified by its own source even if they use well carbon dioxide from an other natural origin. The triangels in Fig. 4 at the upper part of the diagram represent samples which are carbonized by well CO_2 not originating from their own source at the companies source area. The water must be declared as 'carbon dioxide added' neglecting the origin of the gas added, be it from wells or from technical procedures.

3.2. Fertilizer studies using natural $^{15}N/^{14}N$ variation

Vineyards in northern Europe are actively green only from May until October. In wintertime a vineyard consists of resting stems only. During the same period most of the ground water is formed leaching off the nitrate down to the ground water level. In order to keep the nutrients in place the idea was to cover the surface of vineyards with grass or other plants. Legumes would be able to supply the wine plants with air-fixed nitrogen. However, the grass cover also uses the fertilizer nitrogen to built up its own biomass and the fertilizer nitrogen is transported down to the roots of the wine plants in the subsequent year only. Fig. 5 reports results from the biomass of soil covering vegetation and from wine plants as well as from the fertilizer applied and from the soil nitrogen pool. In this case the $^{15}N/^{14}N$ ratio is close to that of the fertilizer applied. The different $^{13}C/^{12}C$ ratios may result from the uptake of carbon dioxide either from the free atmospheric carbon dioxide pool (wine) or from a mixture with the carbon dioxide from soil respiration.

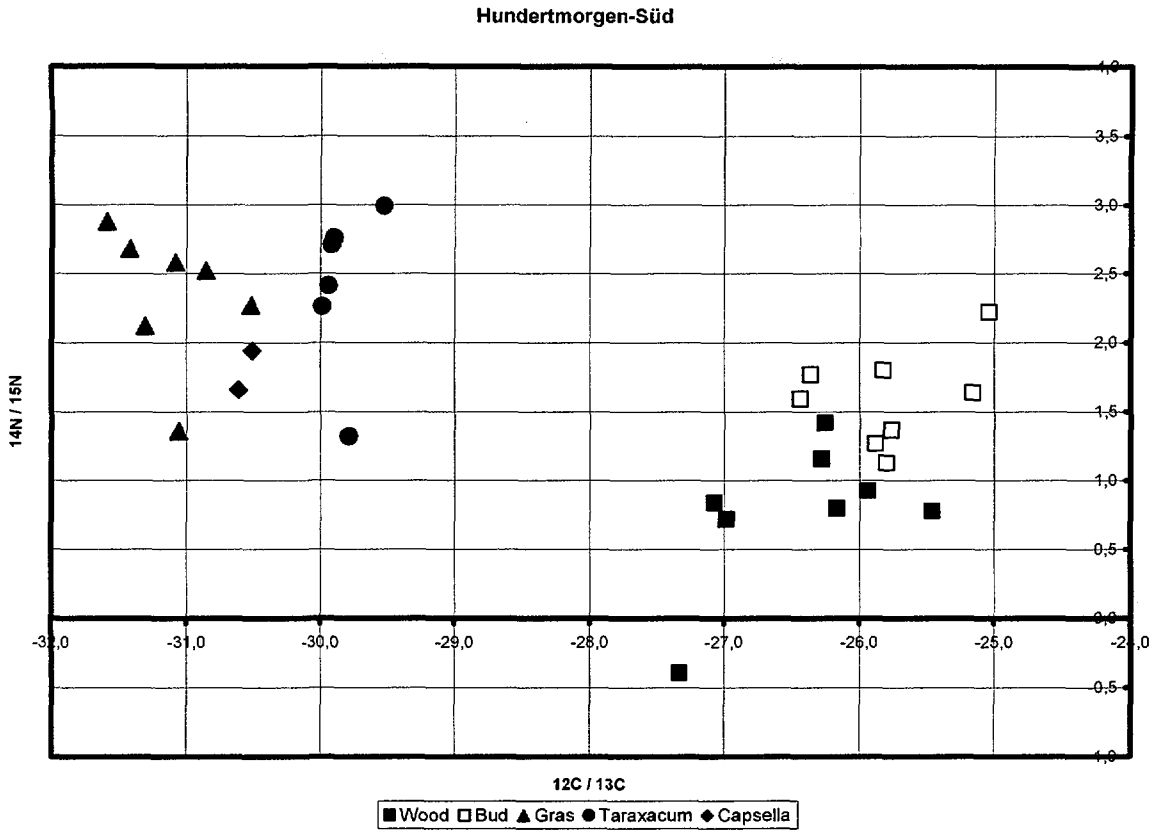

Fig. 5. $^{15}N/^{14}N$ versus $^{13}C/^{12}C$ ratios of soil covering vegetation and of wine plants in a vineyard of Neustadt/Weinstrasse, Palatinate, Germany.

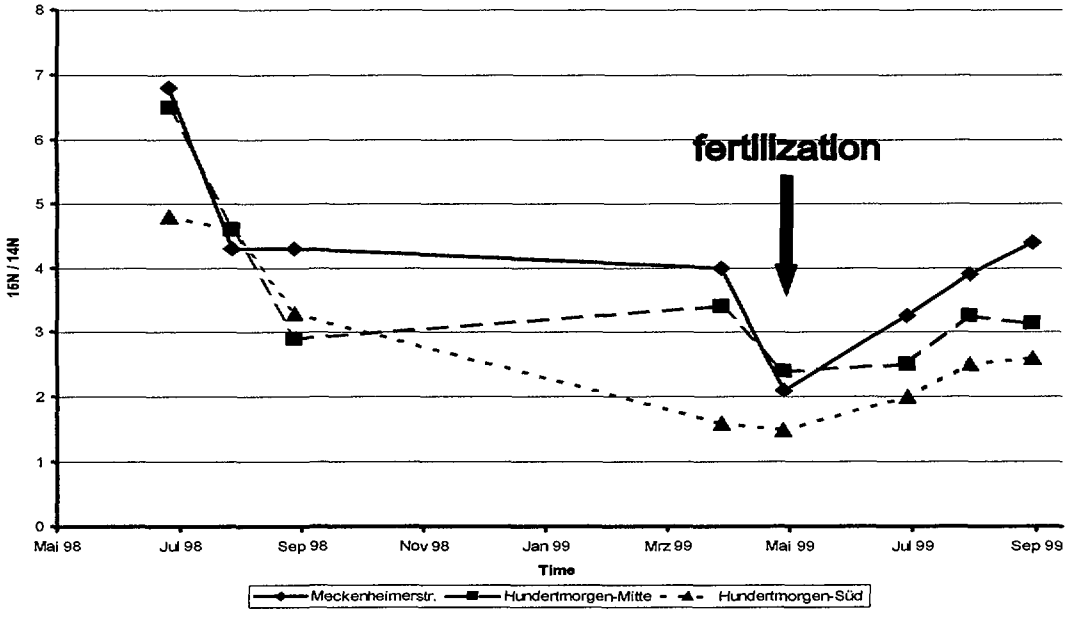

Fig. 6. $^{15}N/^{14}N$ ratio in the soil nitrogen pool and in the corresponding biomass of wine in a vineyard of Neustadt/Weinstrasse, Palatinate, Germany.

Fig. 6 gives an impression of the dynamics of the nitrogen uptake and movement in the soil using the $^{15}N/^{14}N$ data only. Caused by a change in the isotopic composition of the fertilizer the wine plants do not immediately take up the freshly added fertilizer. Rather they mainly utilizes nitrogen from previous fertilizer additions. Until the end of the growth period wine plants get nitrogen from the fertilizer too. Later on they extract nutrients from the soil nitrogen pool again. The soil nitrogen pool tends to more positive $^{15}N/^{14}N$ ratios, possibly caused by an enrichment during previous repeated biochemical turnovers.

4. CONCLUSION

The origin of carbon dioxide added to beverages can be distinguished clearly between natural wells or industrial processes. The relatively large number of products which use carbon dioxide from their own well results from the geographical position of our laboratory which is close to an area of recent volcanic activity (Eifel region, last activity several thousand years ago). A large number of acidic bicarbonate mineral waters points back to the geological history [2–6]. At some places like Maria Laach carbon dioxide is still released continuously to the environment. The method described enables the detection of added industrial carbon dioxide to a product claimed to be of natural origin from a natural well. The method cannot control the use of the carbon dioxide well at the companies facilities until now.

The observation of the $^{15}N/^{14}N$ ratios may become a valuable tool for fertilizer studies without changing the cultivation practice and commercial use. Only the isotopic composition of the fertilizer has to be changed, maybe only the supplier. A convenient label could be set by changing from one fertilizer produced following the Haber-Bosch-procedure (air-N_2) to one of natural origin (e.e. guano). The turnover in a natural ecosystem is a complex process depending on a variety of factors. Therefore conclusions should be drawn carefully. Nevertheless the first summary is that the turnover of fertilizer nitrogen is more complicated than is expected normally, demonstrated by the measurements of the natural variation of the stable isotopes of nitrogen, the $^{15}N/^{14}N$ ratio. "Old" nitrogen seems to be enriched as has been demonstrated previously [7].

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