Historical evolution of analytical methods for nitrate and ammonium isotope measurements

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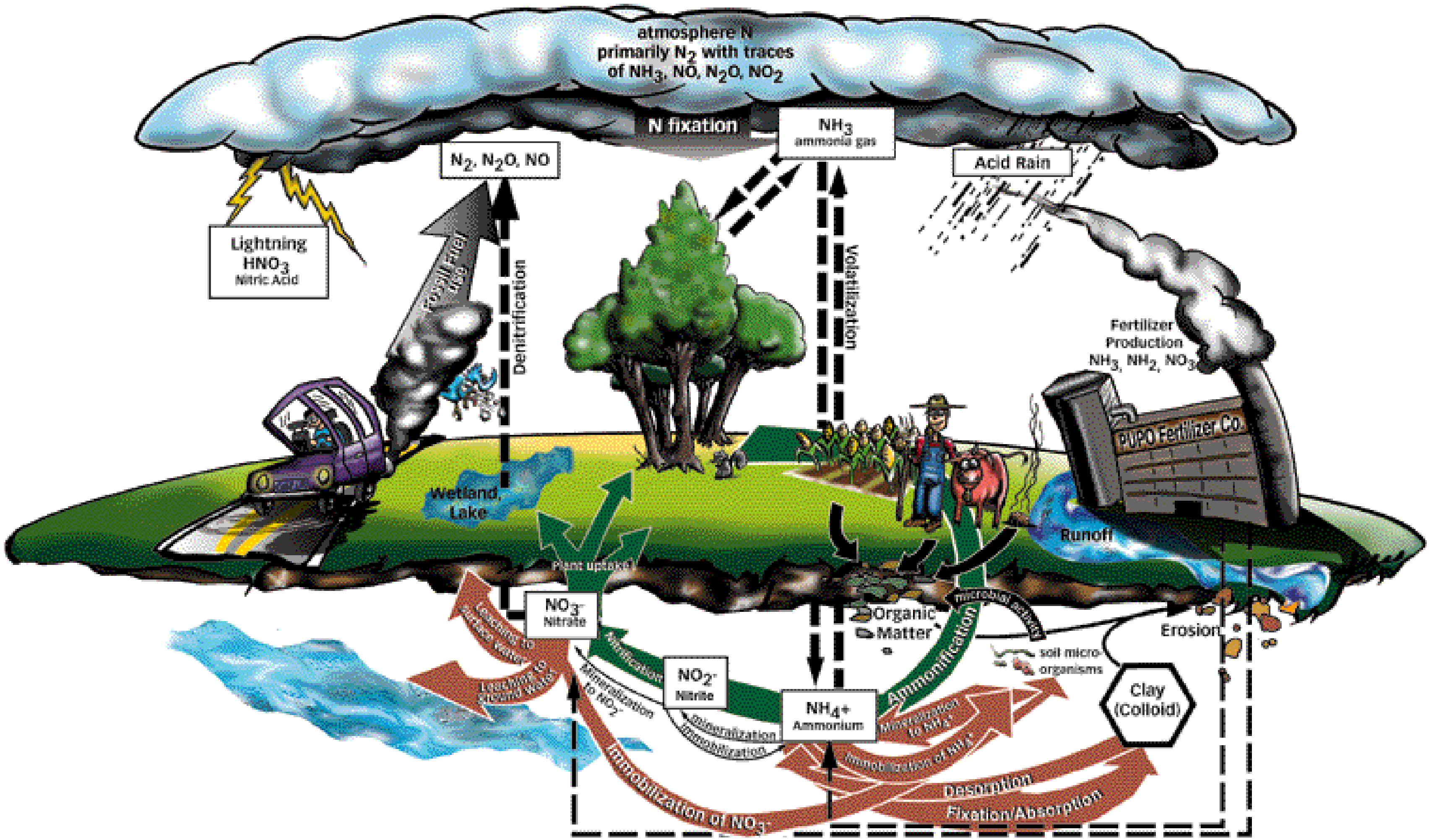
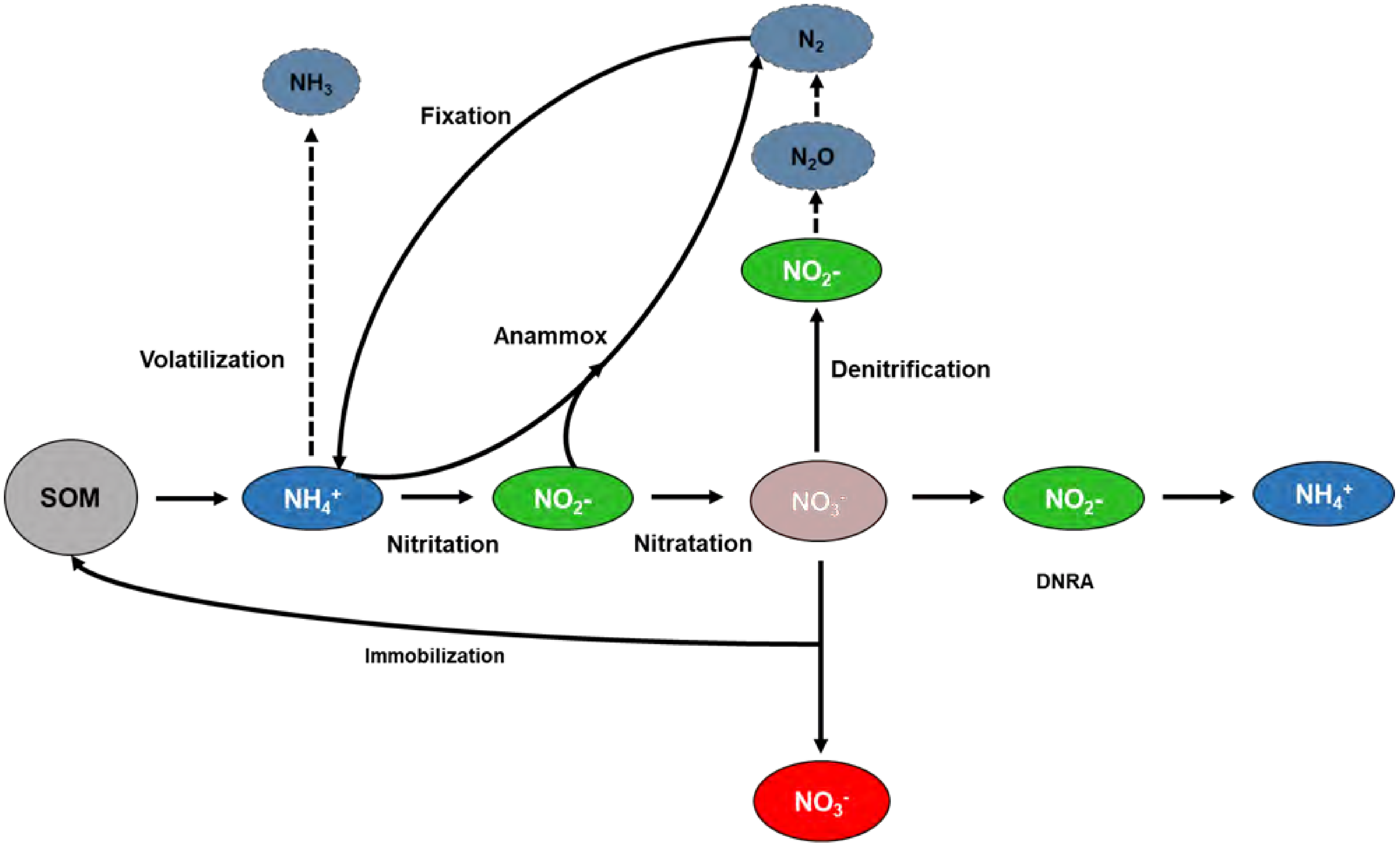


Brevik, 2012

The nitrogen cycle: Sources & Processes



Margalef et al., TrAC 2026

Isotopic Biogeochemistry : integrating tool

Basic Idea:

Isotopic composition of a chemical species at a definite location reflects:

(1) Its various sources

$$\delta_{sample}\,(‰) = \left(\frac{R_{sample} - R_{standard}}{R_{standard}} \right) \times 1000$$

$$\delta = \left[\frac{R_{sample} - R_{standard}}{R_{standard}} \right] . 1000$$

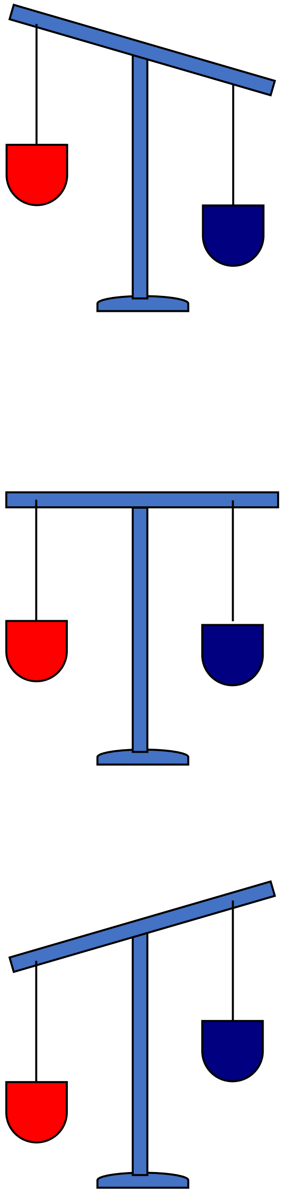
Etalon

$$R = \frac{\text{Quantity of heavy isotope}}{\text{Quantity of light isotope}}$$

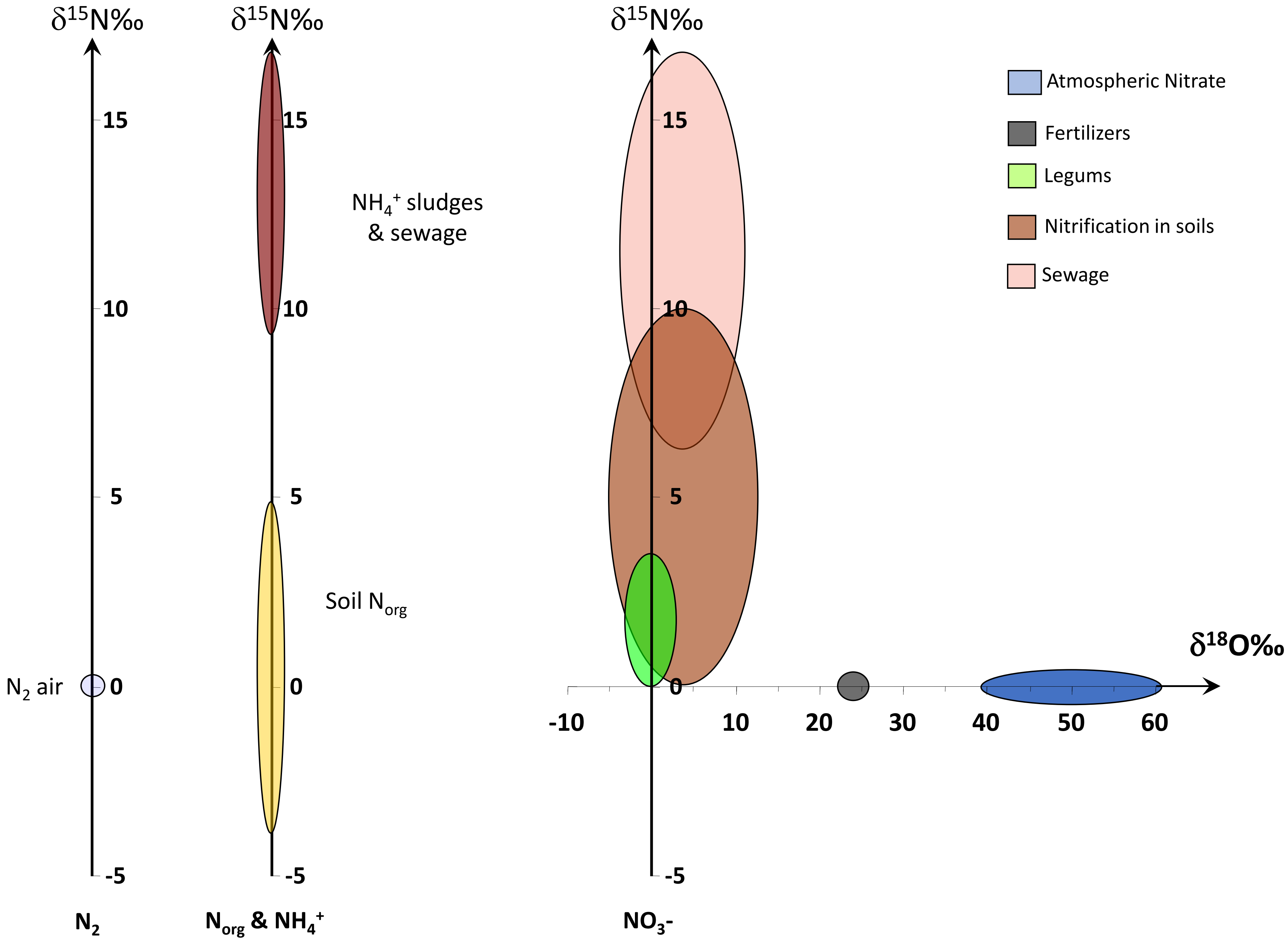
$\delta > 0$ Sample enriched in heavy isotope compared to the standard

0 Standard

$\delta < 0$ Sample depleted in heavy Isotope Compared to the standard



a super-precision balance could measure isotopic composition directly and accurately


δ15N‰
δ15N‰
δ15N‰
15
10
5
0
-5
N2 air
NH4+ sludges & sewage
Soil Norg
Atmospheric Nitrate
Fertilizers
Legums
Nitrification in soils
Sewage
δ18O‰
-10
10
20
30
40
50
60
N2
Norg & NH4+
NO3-

Isotopic Biogeochemistry : integrating tool

Basic Idea:

Isotopic composition of a chemical species at a definite location reflects:

(1) Its various sources

$$\delta_{sample}\,(‰) = \left(\frac{R_{sample} - R_{standard}}{R_{standard}} \right) \times 1000$$

(2) Processes

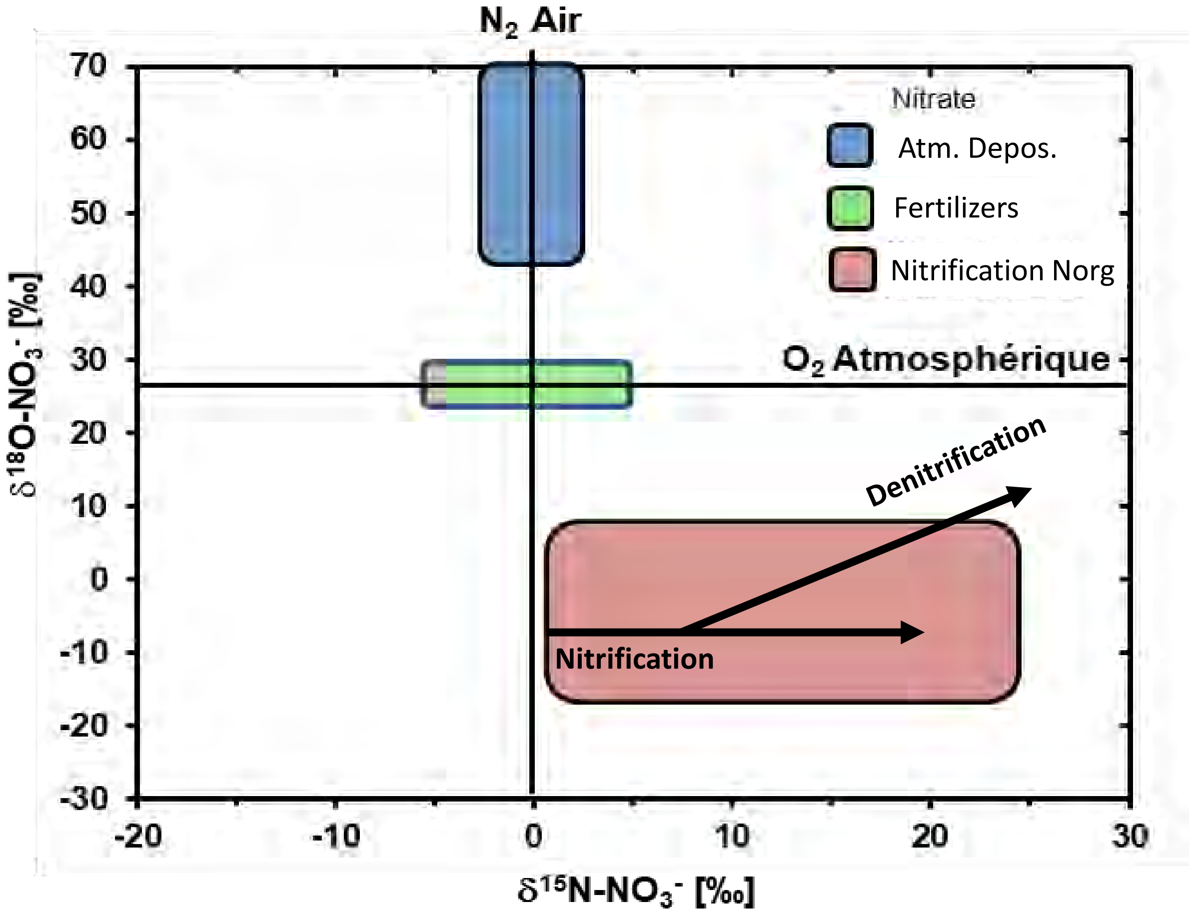
$$\delta = \delta_0 + \varepsilon \ln (C/C_0)$$

$$\Delta\delta = \varepsilon \ln f$$

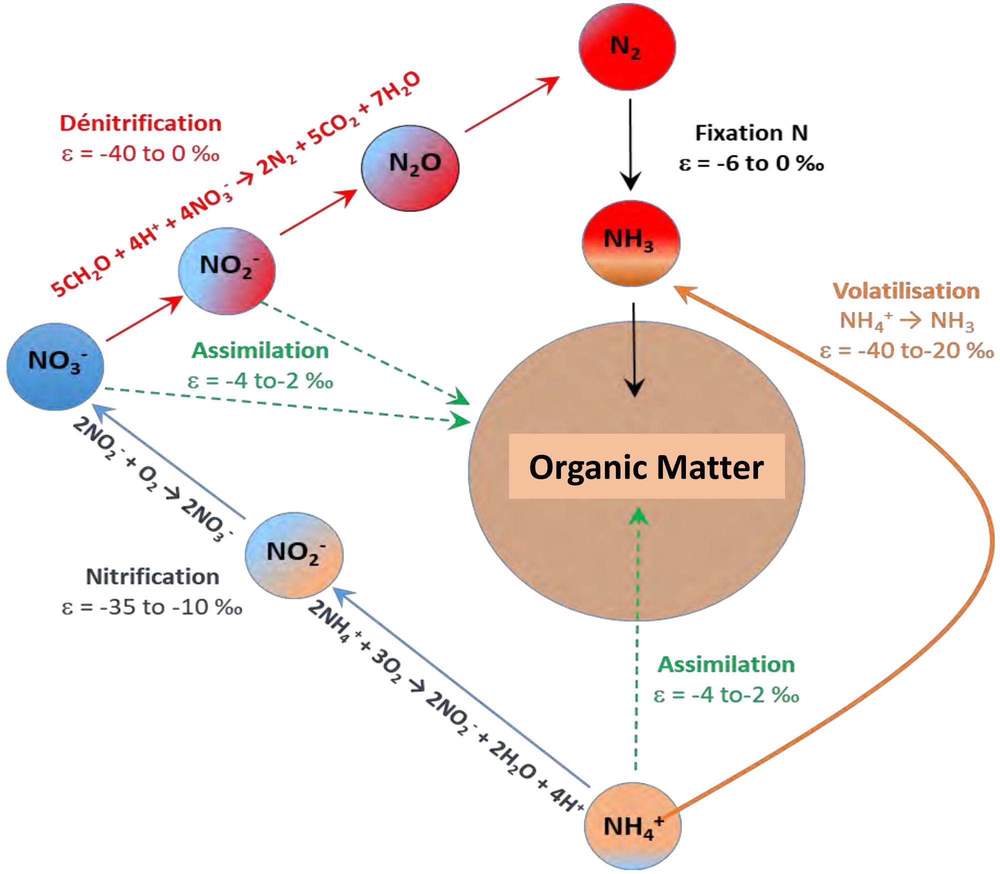
Origin of nitrogen in the catchment

Proof of concept *(Sebilo et al., 2015)*

- Determination of endmembers
- Need to characterize biogeochemical processes which transform nitrogeneous compounds



Elgaouzi et al., Applied Geochem., 2013; Briand et al., Env. Chem. 2013; Sc. Rep., 2017



Sebilo et al., Biogeochemistry, 2003; Env. Chem., 2004; Ecosystems, 2006; Sc. Rep., 2019

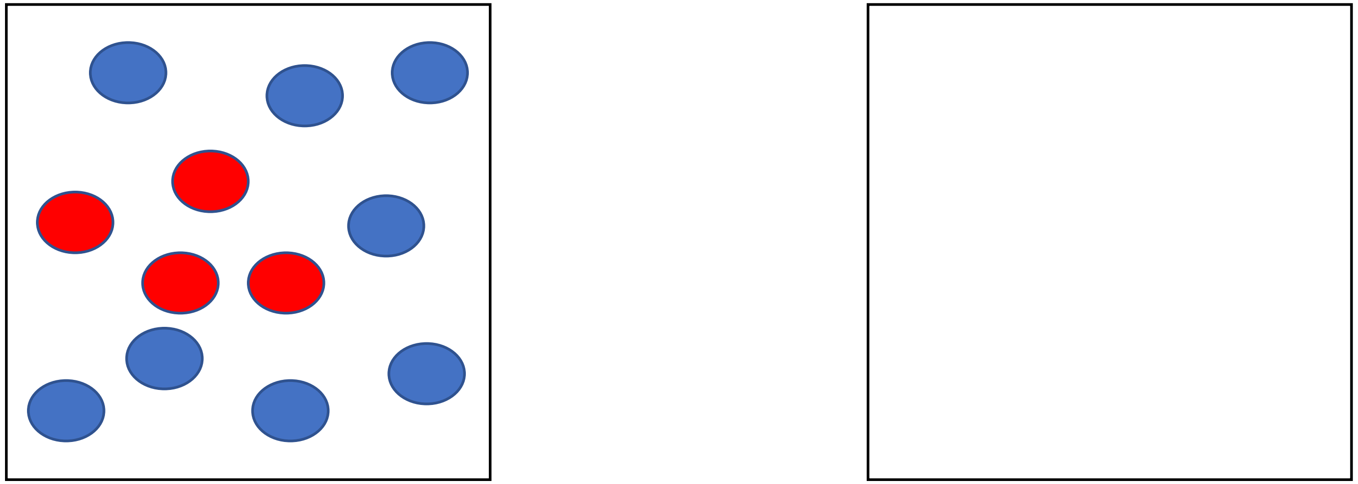
Isotopic Biogeochemistry : integrating tool

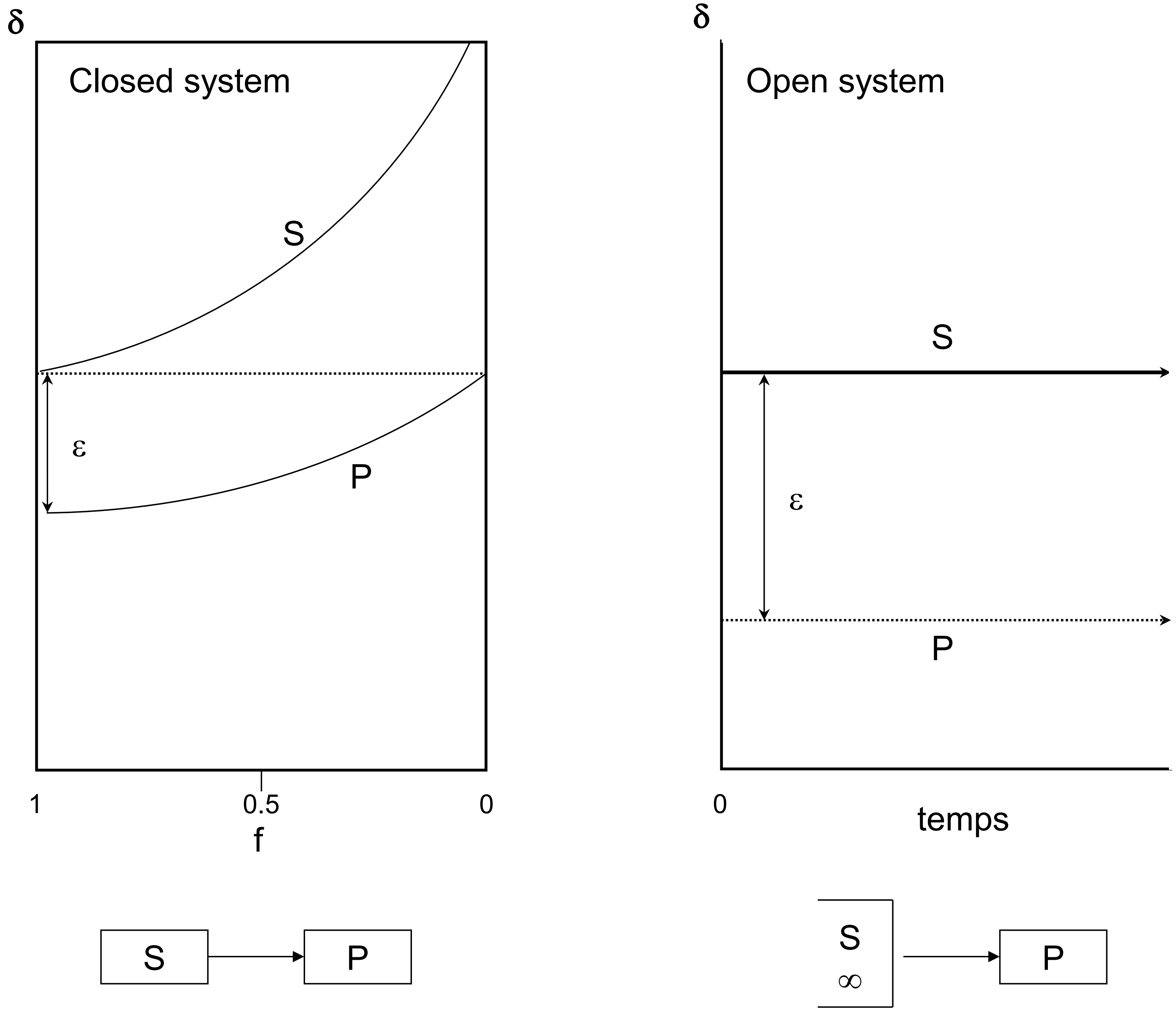
Basic Idea:

Isotopic composition of a chemical species at a definite location reflects:

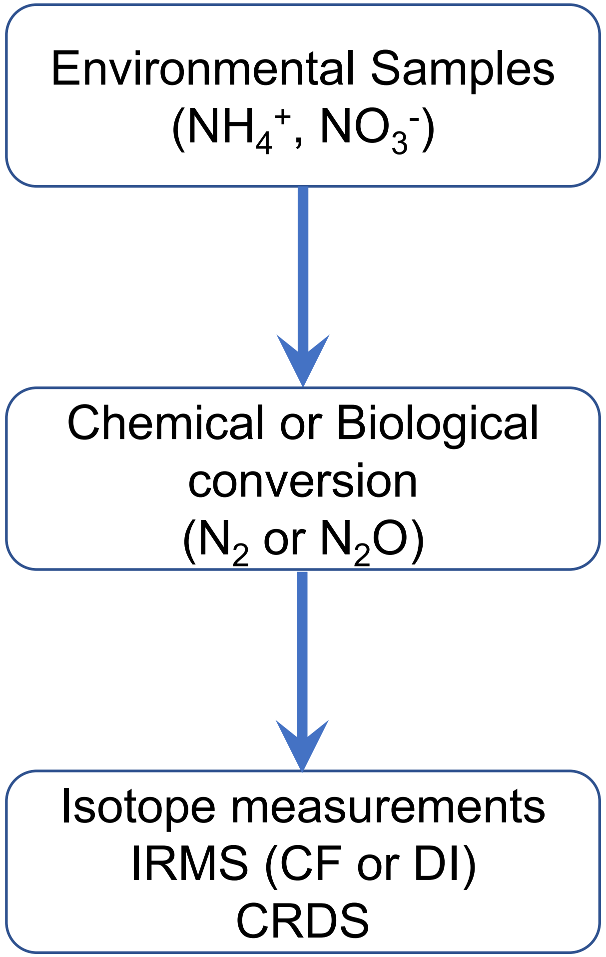
1. **Its various sources**
2. **the processes which affects it**

Substrate → Product




δ
Closed system
S
ε
P
1
0.5
0
f
S
P
δ
Open system
S
ε
P
0
temps
S
∞
P

Analytical Challenges in NH_4^+ and NO_3^- isotope measurements



Additional analytical challenges

- **Very low concentrations**
few µg N L^{-1} (rainwater, ice) to a few mg N L^{-1}

- **Complex environmental matrices**
freshwater, seawater, ice, dissolved organic matter

- **Dual isotopic target**
simultaneous determination of $\delta^{15}N$ and $\delta^{18}O$

- **Limited sample volumes**
precious samples (ice cores, extraterrestrial material)

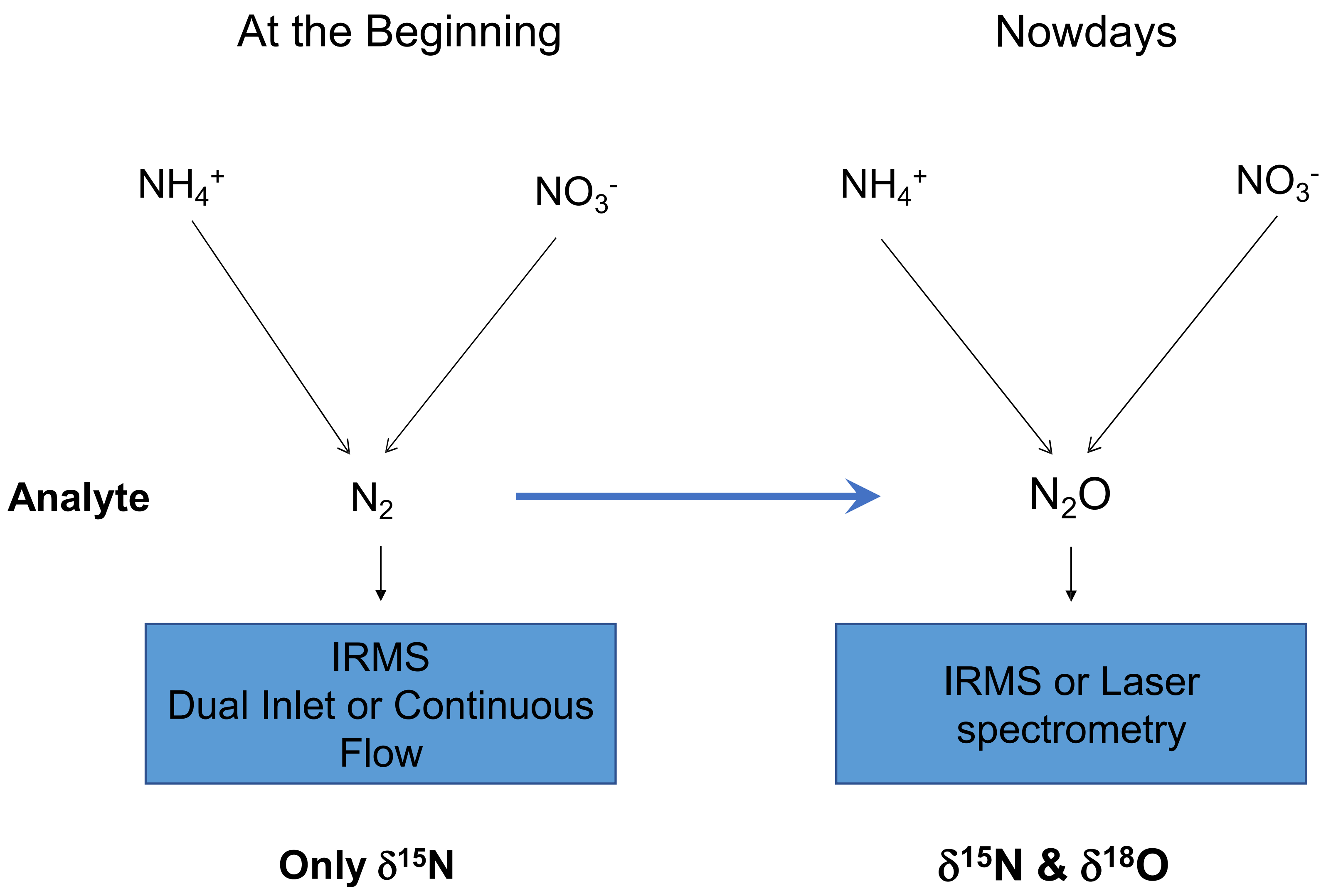
- **Potential interferences**
NO_2^-, dissolved organic matter, salts

Quantitative conversion is required to avoid isotopic fractionation

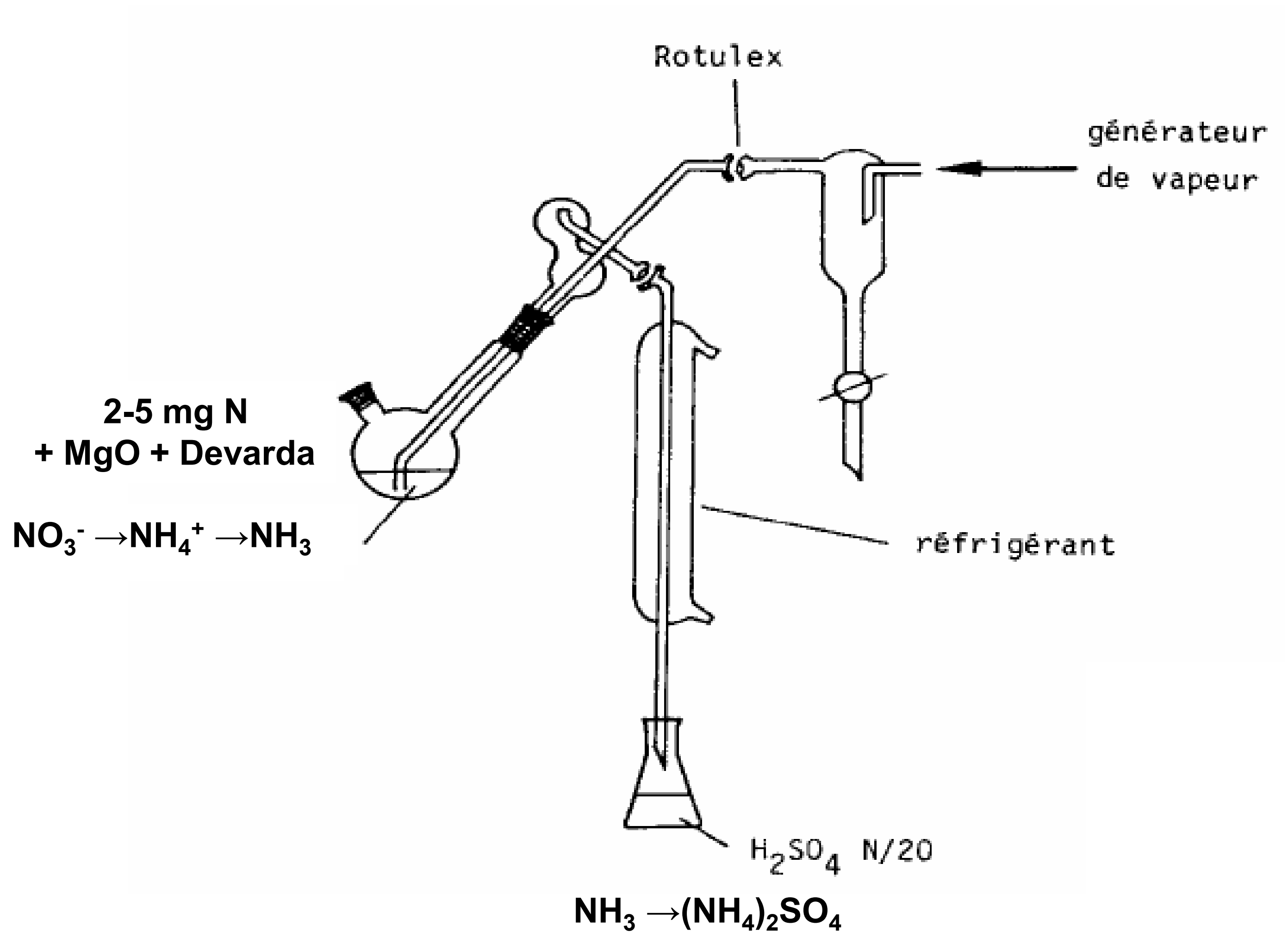
These analytical constraints have driven the development of multiple preparation methods for nitrate and ammonium isotope analysis.

Isotope measurements

$\delta^{15}N$-NH_4^+, $\delta^{15}N$ & $\delta^{18}O$-NO_3^-



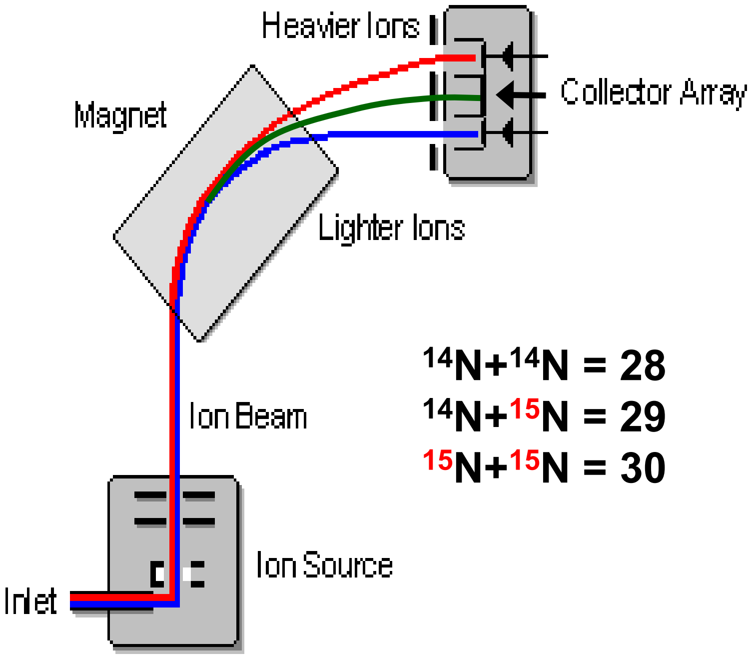
Kjeldahl Distillation: The Last millenium?



- High volumes, 2-3 days of evaporation
- 1 sample/30mn
- Evaporation of ammonium sulfate : 12h

Mariotti et al., 1978

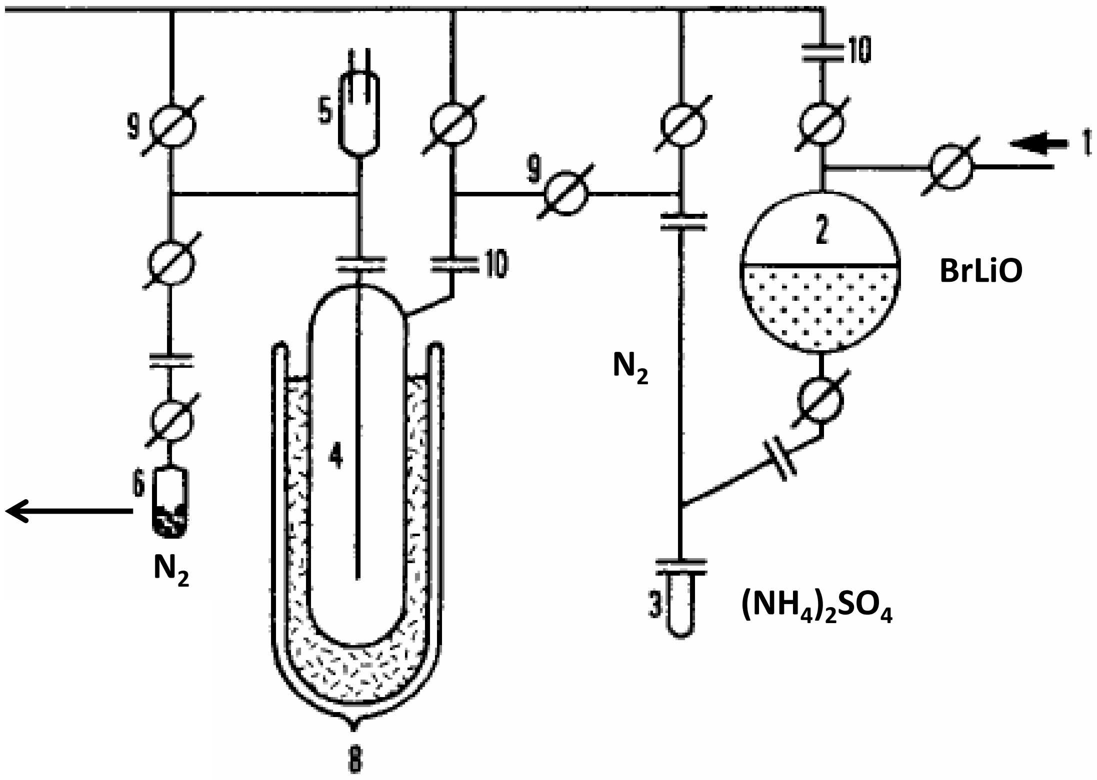
Oxydation with Lithium hypobromite



$^{14}N+^{14}N = 28$

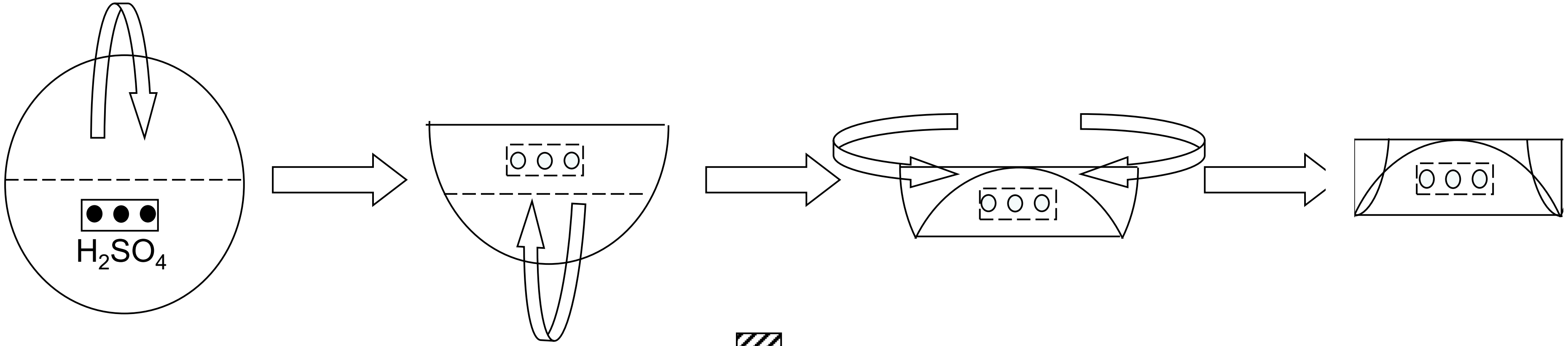
$^{14}N+^{15}N = 29$

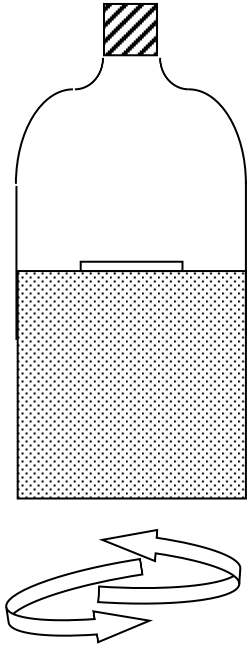
$^{15}N+^{15}N = 30$



- 1 oxydation/30mn
- Isotope measurement/30mn; Dual Inlet
- Robustness and cheap ($\delta^{15}N-NH_4^+$, $\delta^{15}N-NO_3^-$)
- Precision ± 0,2‰

Microdiffusion: A new Millenium





Devarda
NaOH

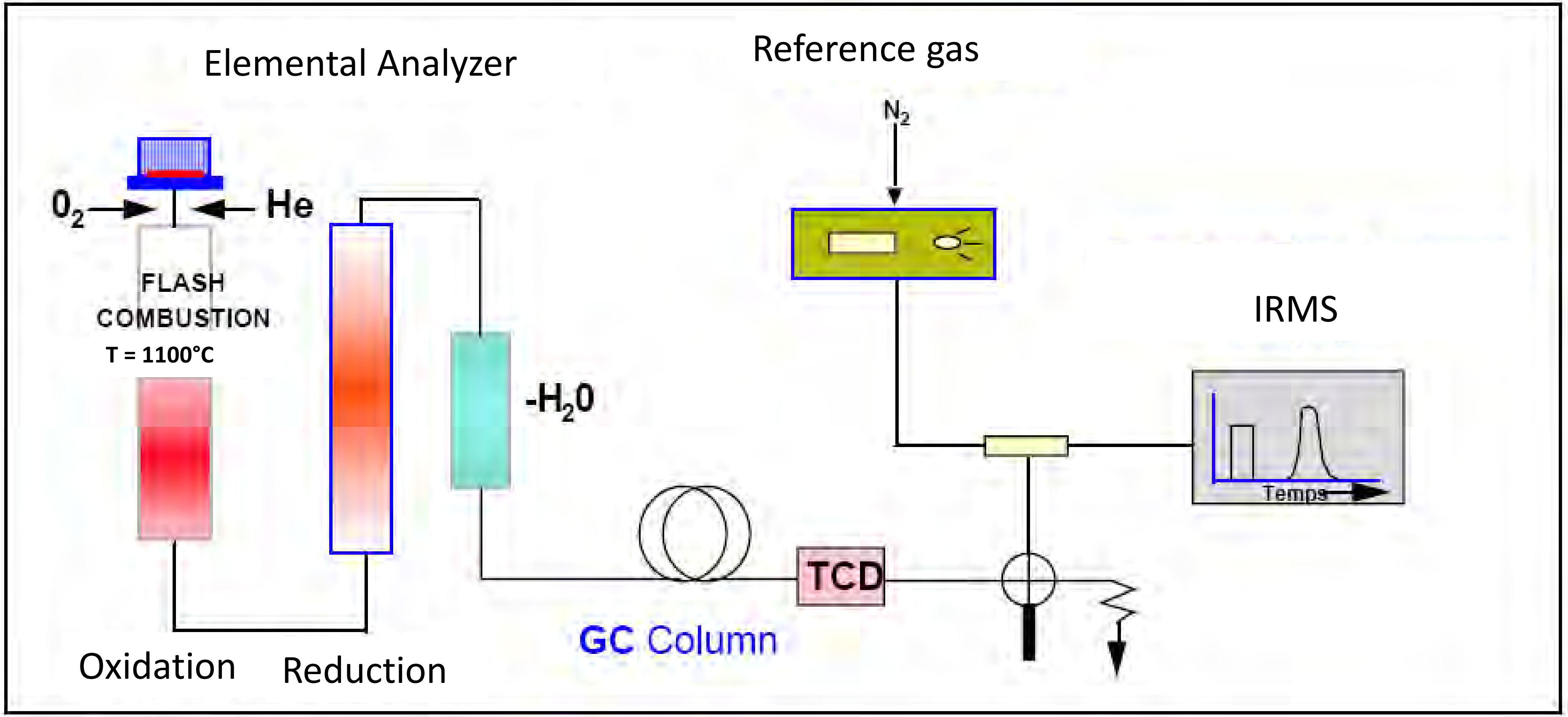
$NO_3^- \rightarrow NH_4^+ \rightarrow NH_3 \rightarrow (NH_4)_2SO_4$

1 week

- 150 µg N
- Preparation of filters 1 day (15 filters)
- 1 week of stirring
- 3 days of freeze drying
- Measurement on EA-IRMS

Sebilo et al., 2004

Continuous Flow - IRMS

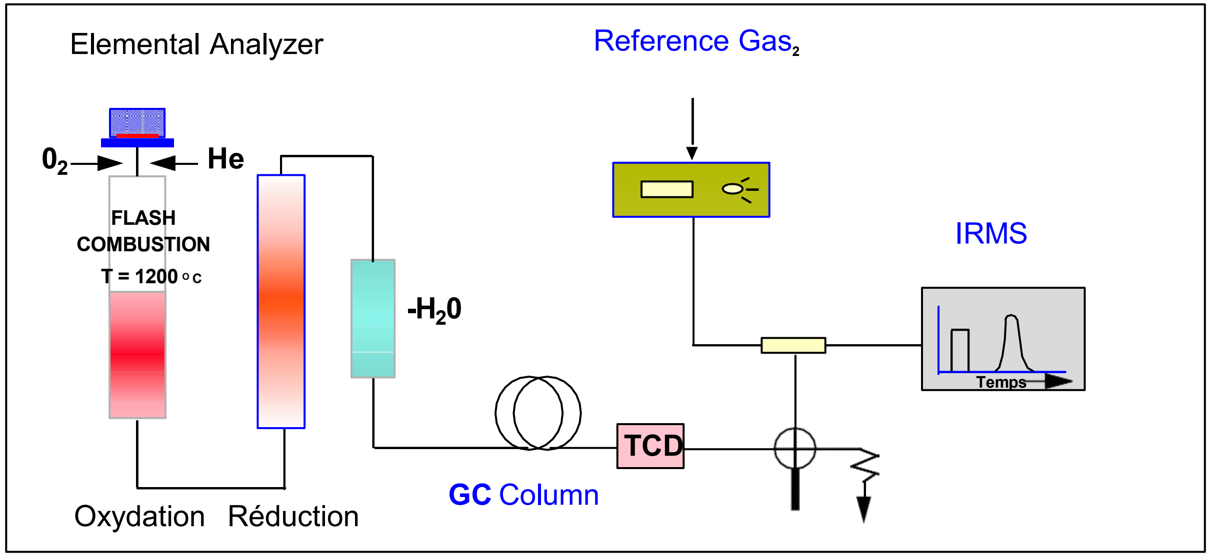


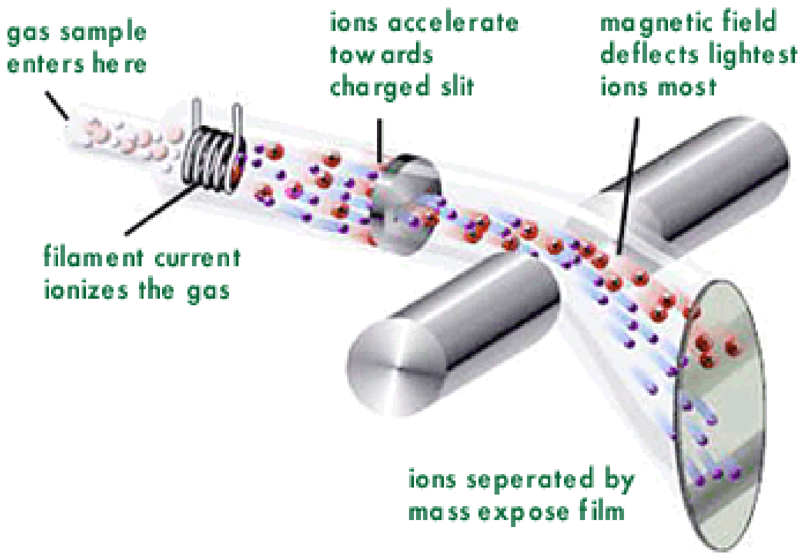
- Automatic, fast (30’)
- Good accuracy

But

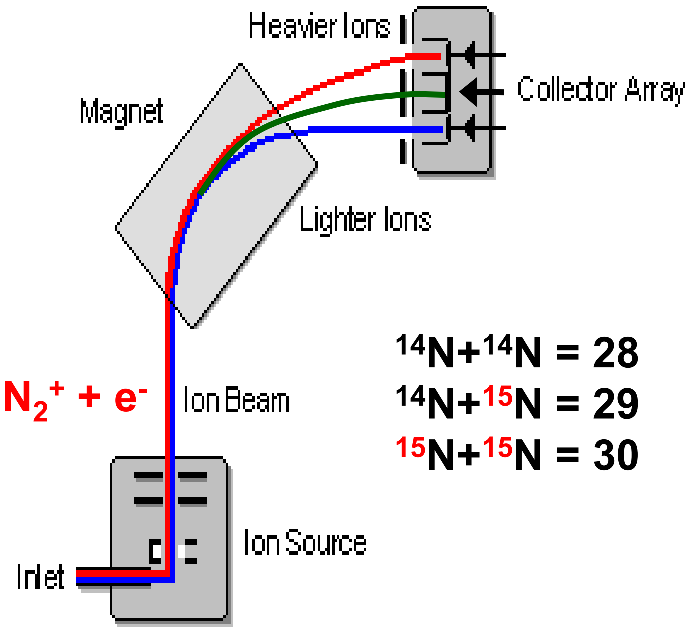
- More expensive than kjeldahl distillation (30€ vs. 10€)
- No $\delta^{18}O\text{-}NO_3^-$

EA - IRMS





$N_2 \rightarrow N_2^+ + e^-$



$^{14}N + ^{14}N = 28$
$^{15}N + ^{14}N = 29$
$^{15}N + ^{15}N = 30$

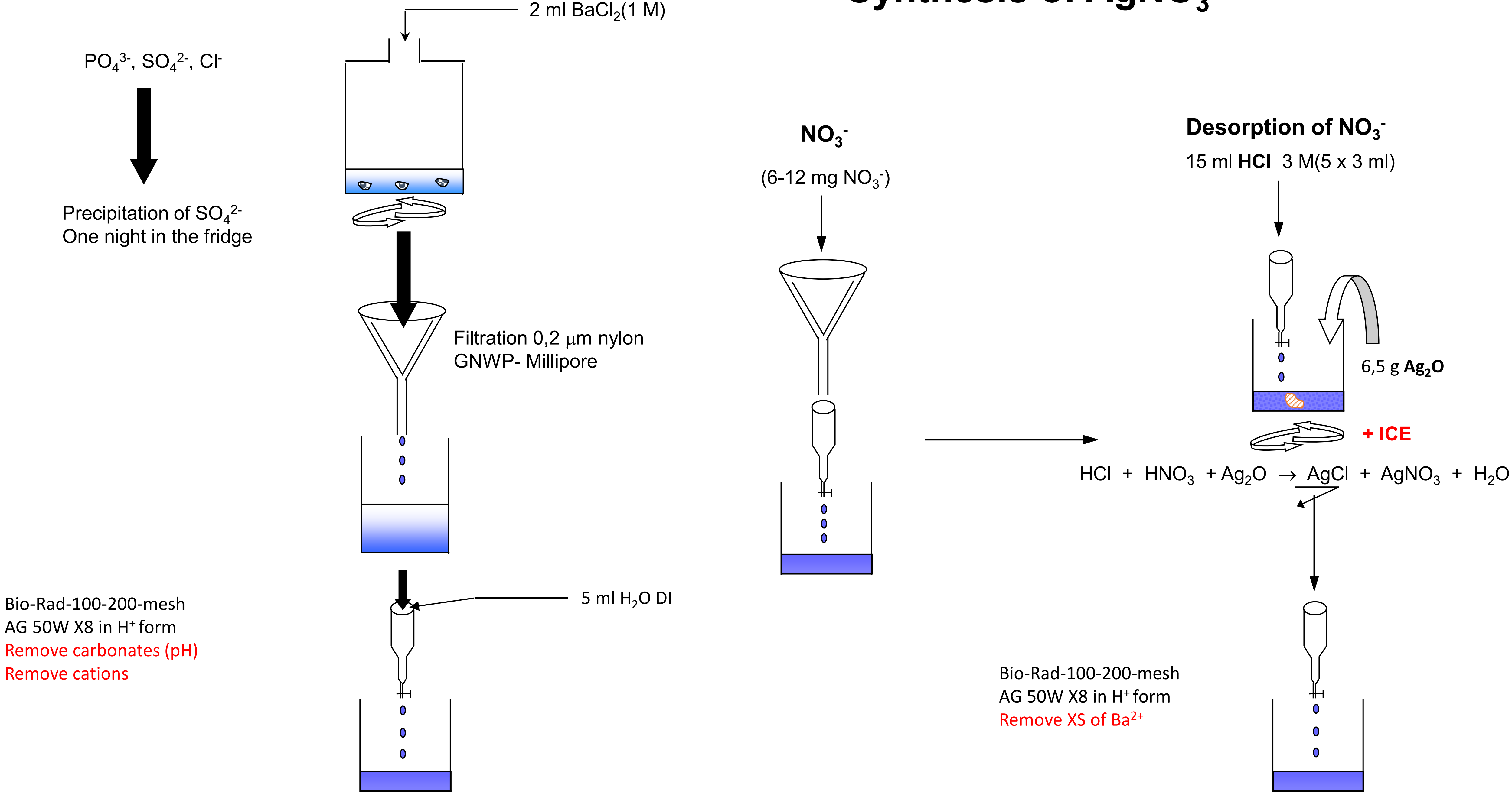
- Automatic, fast (30’)
- Good accuracy

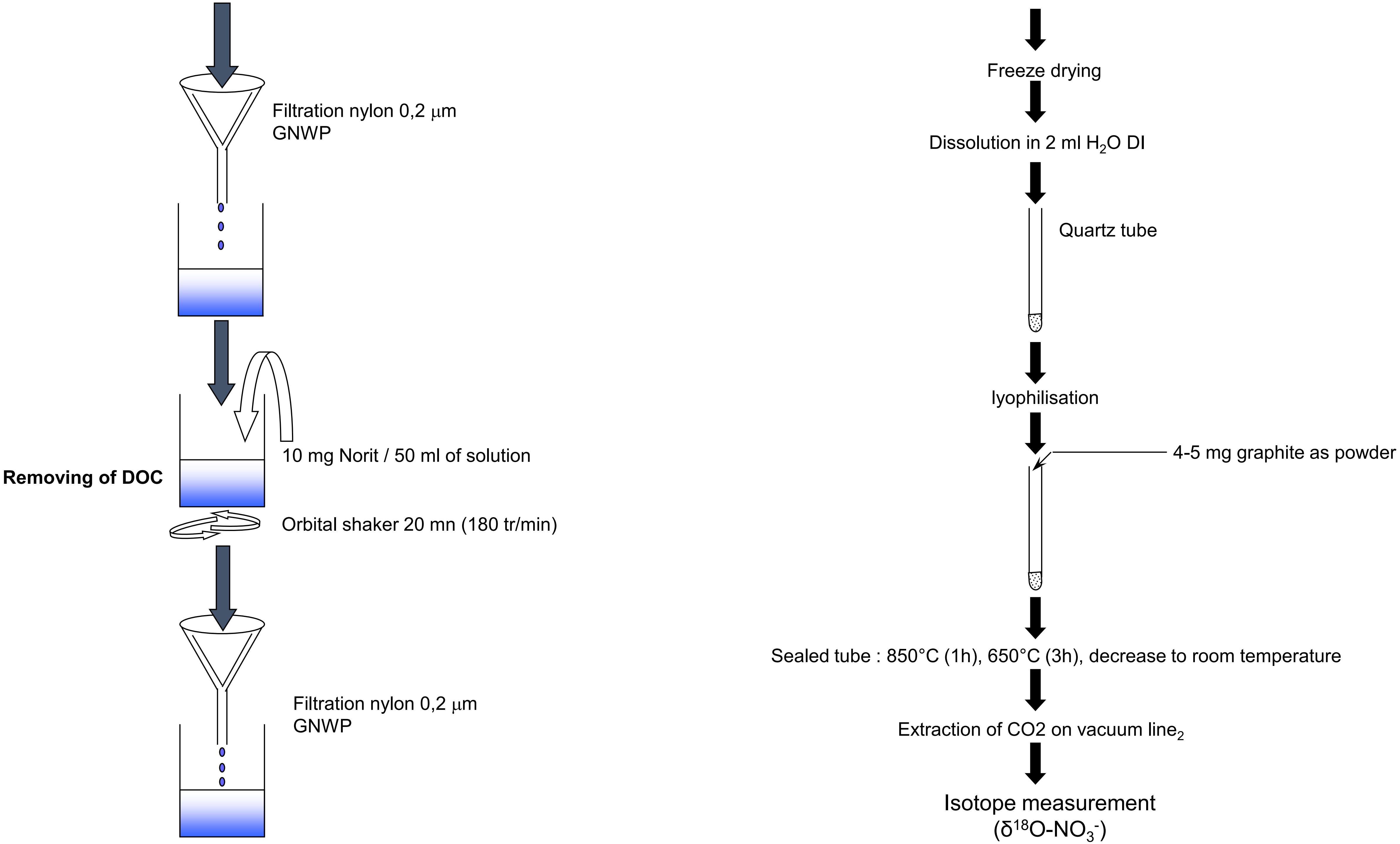
But

- More expansive than kjeldahl distillation (30€ vs. 10€)

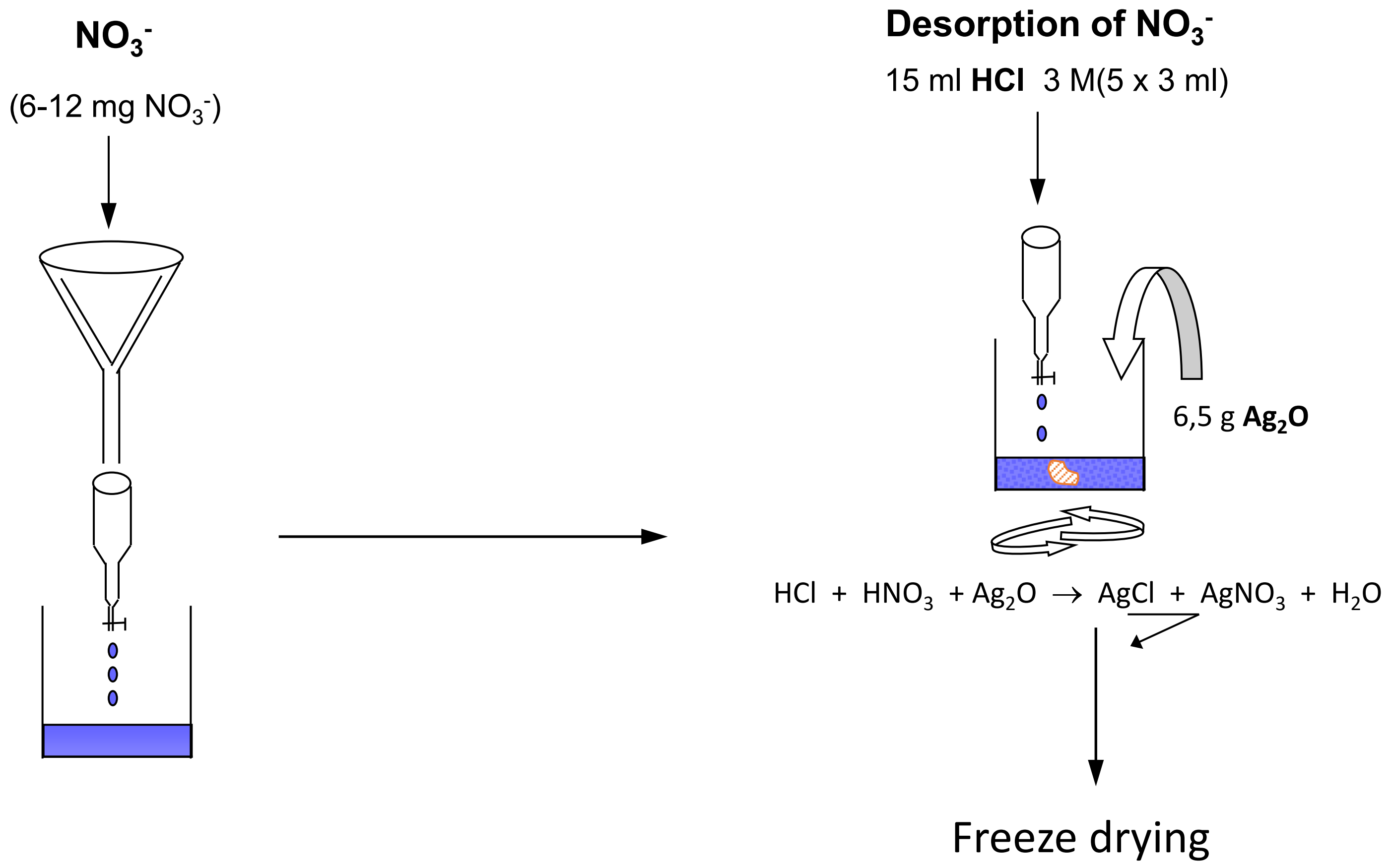
- No $\delta^{18}O\text{-}NO_3^-$

Synthesis of $AgNO_3$




Filtration nylon 0,2 μm
GNWP
Removing of DOC
10 mg Norit / 50 ml of solution
Orbital shaker 20 mn (180 tr/min)
Filtration nylon 0,2 μm
GNWP
Freeze drying
Dissolution in 2 ml H2O DI
Quartz tube
lyophilisation
4-5 mg graphite as powder
Sealed tube : 850°C (1h), 650°C (3h), decrease to room temperature
Extraction of CO2 on vacuum line2
Isotope measurement
(δ18O-NO3-)

Synthesis of $AgNO_3$: ^{15}N & ^{18}O, not simultanously

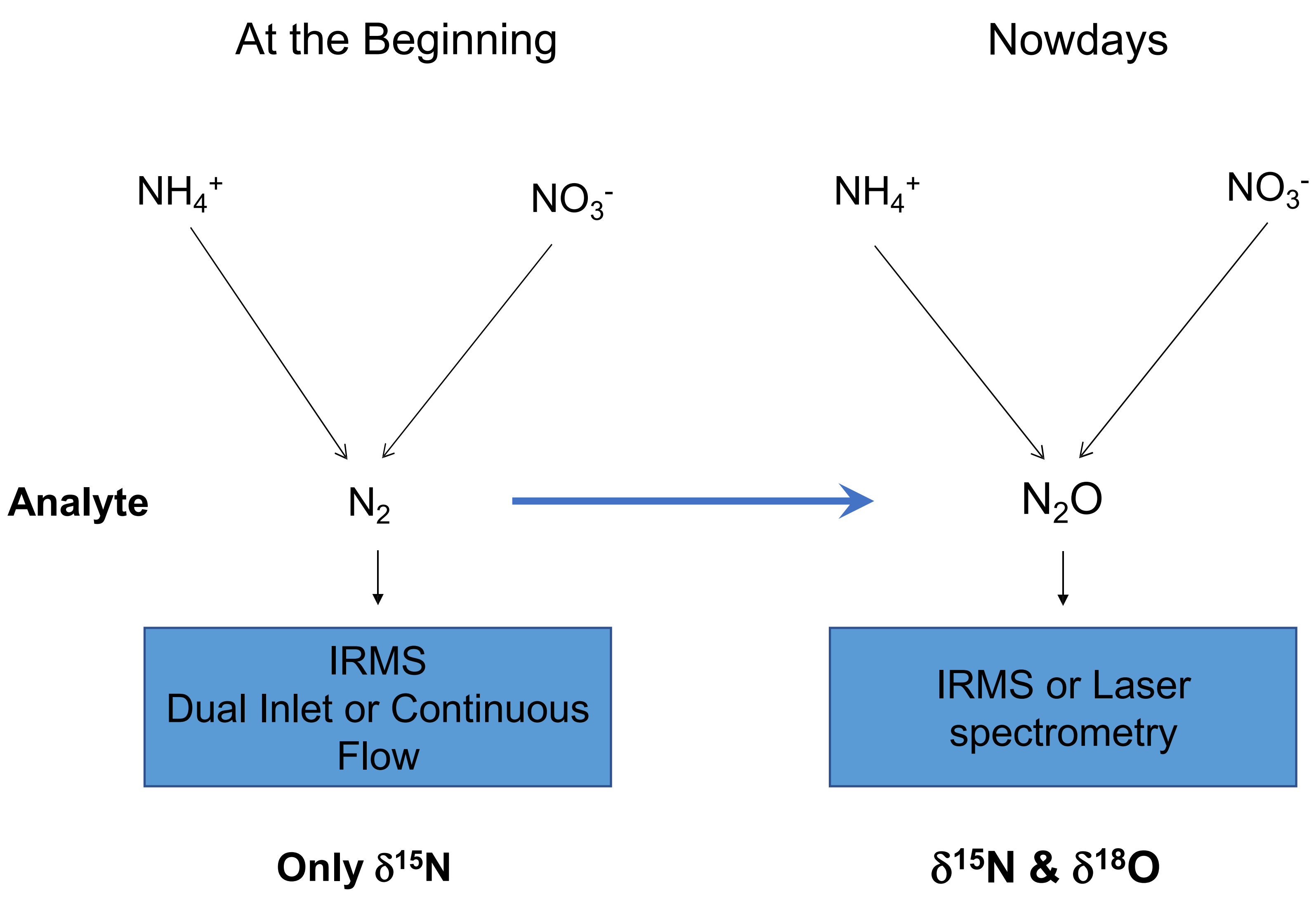


- Hard work; 2 days for 15 $AgNO_3$
- Measurement of $\delta^{15}N$-NO_3^- (EA-IRMS)
- Measurement of $\delta^{18}O$-NO_3^- (dual inlet or pyr-IRMS)

2 setups & difficult for sea waters,
low nitrate concentrations and dissolved Organic Matter

Isotope measurements

$\delta^{15}N$-NH_4^+, $\delta^{15}N$ & $\delta^{18}O$-NO_3^-



Bacterial method (Sigman et al., 2001)

NO_3^- ⟶ NO_2^- ⟶ N_2O ⟶ N_2 Denitrification

Pseudomonas aureofaciens

NO_3^- ⟶ NO_2^- ⟶ N_2O ⊖ ⟶ N_2

N_2O Accumulation with yield of NO_3^- conversion ≈ 100 %

No Isotopic fractionation = good method

$\delta^{15}N$ & $\delta^{18}O$ on the same time!!!

100 analysis a week

Need to remove NO_2^-

Bacteria growth/activity

Chemical conversion ($NO_3^- \rightarrow NO_2^- \rightarrow N_2O$)

1. Nitrate reduction (10 ml at 40 µM)

- Activated column of cadmium

- Reduction of nitrate into nitrite

- **Yield of reduction**

- 15 samples/days

2. Reduction of nitrite with NaN_3 (15 nmole of N_2O)

- Measurement of both $\delta^{15}N$ & $\delta^{18}O$

- 15 samples/days (standards & samples)

- Sequantial method with test of robutness at each step

- $\delta^{15}N$ & $\delta^{18}O$ of NO_2^-

Cd

NaN_3 explosive

McIlvin & Altabet, 2005; Semaoune et al., 2012; Sebilo et al., 2019

Chemical conversion (NH_4+ → NO_2^- → N_2O)

1. NH_4^+ conversion (20 ml at 10 µM)

$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_20$$
$$Br_2 + 4OH^- \rightarrow 2BrO^- - + 2H_2O$$

$$3BrO^- + NH_4^+ + OH^- \rightarrow NO_2^- + 2H_20 + 3Br^-$$

2. Reduction of nitrite with NaN_3 (15 nmole of N_2O)

- Measurement of both $\delta^{15}N$ & $\delta^{18}O$

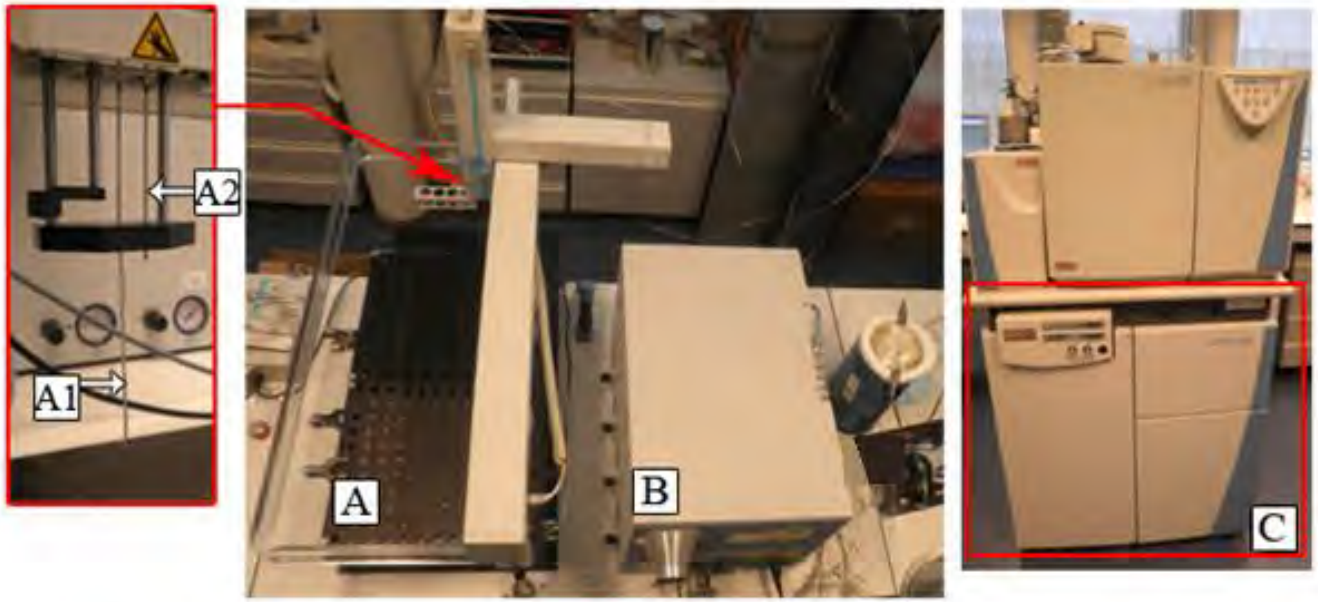
- 15 samples/days (standards & samples)

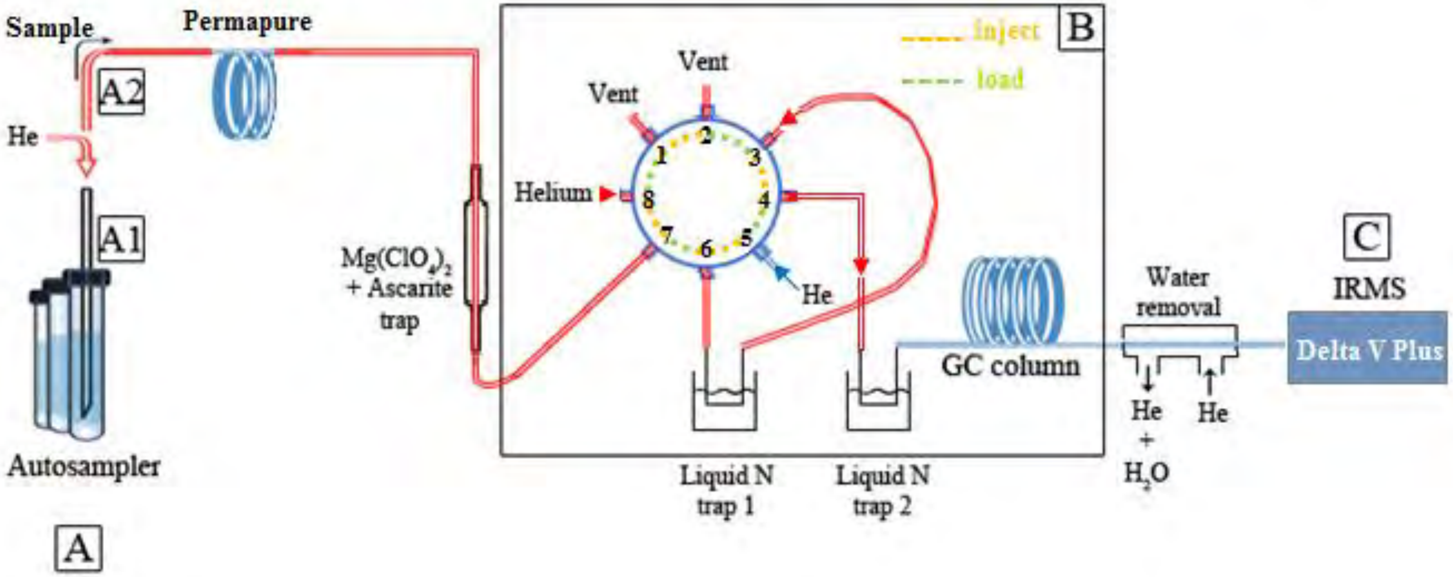
- Sequantial method with test of robutness at each step

- $\delta^{15}N$ & $\delta^{18}O$ of NO_2^-

First production of Br_2 (acidic conditions) → strong variations of pH
Précipitation of the goechemical matrix

GasBench – Denitrification Kit + Delta V Plus





	Steam Distillation	Microdiffusion	AgNO3	N_2O Chem. or Biol. Conversion
Amount	2 à 5 mg N 	150 µg N 	300 µg to 1 mg N 	5 to 20 µg N 
SD ^{15}N (‰)	0.2	0.2	0.3	0.2
SD ^{18}O (‰)	-	-	0.5	0.5
Time for 15 samples (days)	5	10	7	1
Cost/ sample	10 €	30 €	75 €	5 €

Comparative table

Methods increasingly sensitive : from mg N → µg N → ngN (Sampling)

Progressive access to $\delta^{18}O\text{-}NO_3^-$, key information for tracing sources and processes

But each method remains relevant depending on the matrix and the scientific question

Methods increasingly faster and less labor-intensiveTi(III) + CRDS : promising for challenging matrices and field deployment

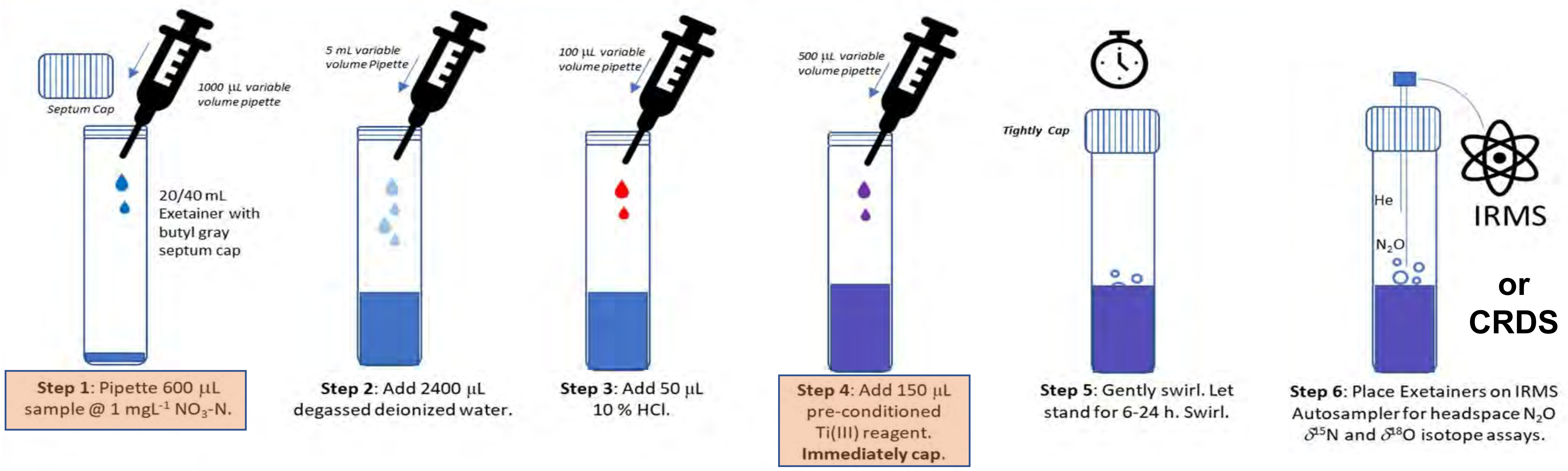
The New Titanium (III) Method; Altabet et al., 2019

Generalized Chemical Reduction Pathway

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O/NH_4^+$$

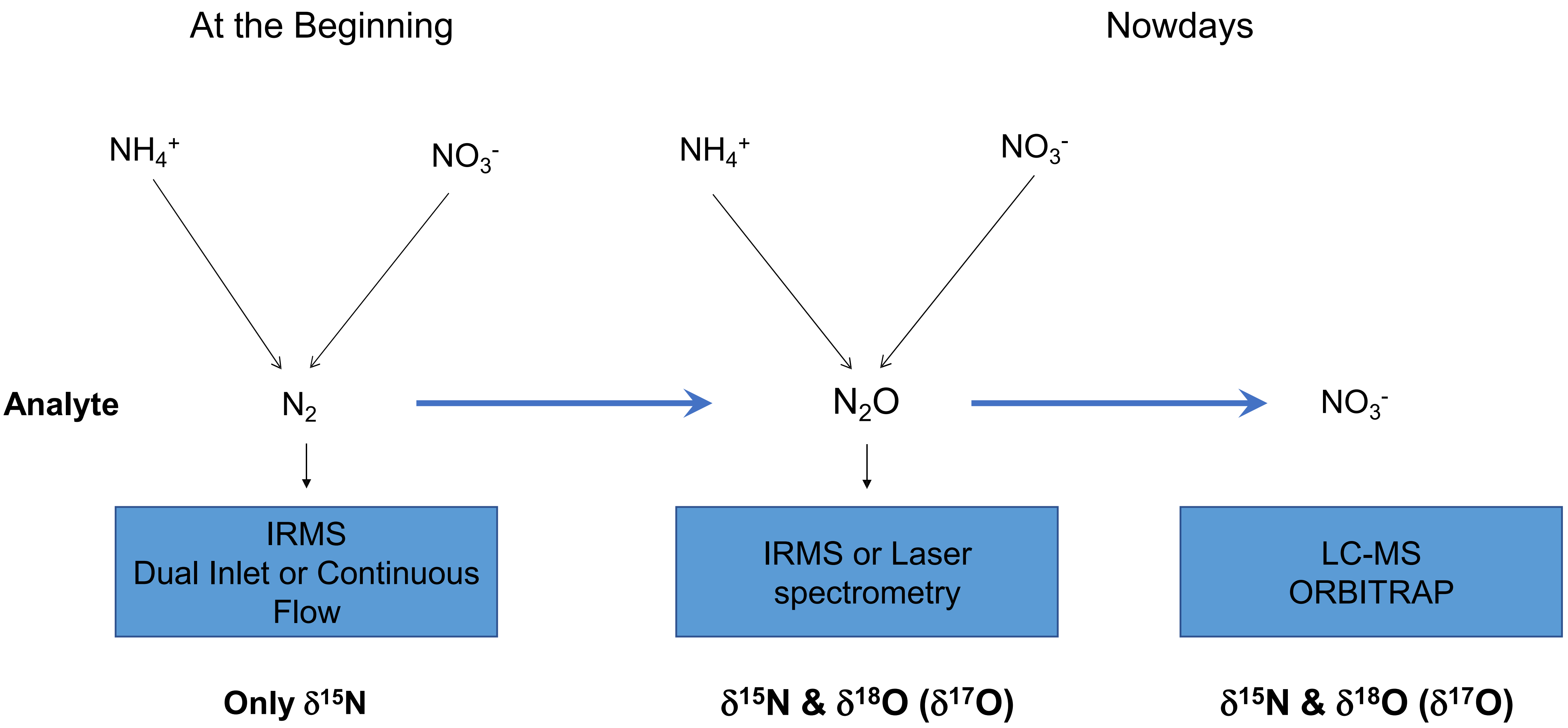
In the presence of Ti(III) reducing reagent the reaction goes to NH_4^+ under basic conditions. This was the basis for nitrate analyzers in the 1980-90s

Under acidic conditions the reaction terminates at N_2O (mostly) – Stedman (1963) – hence can we do isotopes?



Isotope measurements

$\delta^{15}N$-NH_4^+, $\delta^{15}N$ & $\delta^{18}O$-NO_3^-



From environmental nitrogen to isotope-based process understanding

ENVIRONMENTAL SYSTEM

NH_4^+ NO_3^-

Low concentrations - Complex matrices

ANALYTICAL PREPARATION

Chemical or biological preparation
Quantative conversion

IRMS/CRDS measurement
$\delta^{15}N$ & $\delta^{18}O$

ENVIRONMENTAL INTERPRETATIONS

N sources
and
Biogeochemical Processes