

***Possibilities and limitations of using isotope & multi-element techniques to distinguish between 'Ecological' and 'Conventional' agricultural practices***

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- 
- Background
    - social & economic context
    - scientific context
  - Results
    - Stable nitrogen isotope analysis
    - Trace element analysis
  - Conclusions
  - Challenges for the future

- 
- Consumer awareness
    - Mistrust of ‘conventional’ intensive farming methods – pesticides, herbicides, synthetic fertilisers, veterinary drugs ....
    - Purported health benefits
    - Concerns over animal welfare
    - Environmental concerns
    - Food miles

- 
- The global organic food market grew by 13.6% in 2006 to reach a value of \$36.7 billion.
  - In 2011, the market is forecast to have a value of \$67.1 billion, an increase of 83% since 2006.
  - Fruit and vegetable sales form the most lucrative segment of the market, accounting for 39.5% of the market's value.
  - The largest organic food market is the Americas, which accounts for 49.7% of the global market's value.

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# The higher production costs associated with Organic agriculture

- Less intensive methods
- Lower yields
- Greater care of the farm environment
- Segregation of Organic Produce

## = Premium retail prices

- Price differentials between conventional and Organic crops can vary as much as 20 to 250% depending on crop type e.g. wine, coffee and tea

## = Economic incentives exist to mis-describe 'conventional' produce as 'Organic/Biological/Ecological'

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# Organic Farming

- Organic farming severely restricts the use of artificial chemical fertilisers and pesticides.
- Organic farmers rely on developing a healthy, fertile soil and growing a mixture of crops.
- Management practices which sustain soil health and fertility.
- The use of natural methods of pest, disease and weed control.
- High standards of animal welfare.
- Low levels of environmental pollution.
- Enhancement of the landscape, wildlife and wildlife habitat.
- The prohibition of all genetically engineered food and products.

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## Project Aim

To discover whether it is possible to discriminate between organically and conventionally grown crops using stable isotope and multi-element analysis.

## Project Raison d'être

- **Prevention of food fraud**

Help prevent the mislabelling of conventionally cultivated produce as 'organic' by developing analytical methods to check off-the-shelf products

- **Protect the interests of the consumer**
- **Protect the interests of honest growers and traders**

# Organic Fraud



“Can nitrogen isotope and TE analysis help us detect conventional crops that have been labelled as Organic?”

Introduction

**Stable isotopes**

Fertiliser data

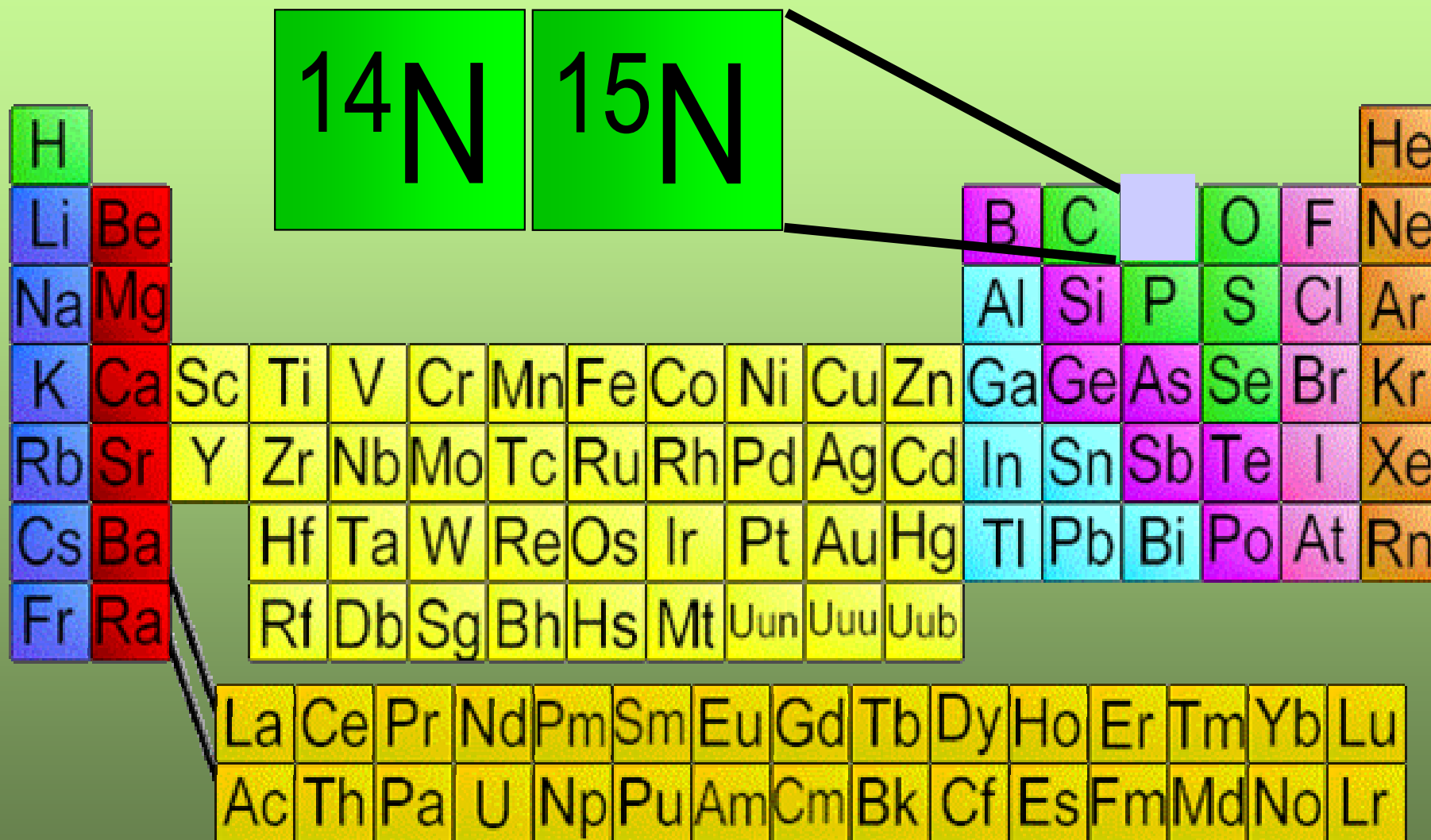
Controlled cultivation experiments

Baseline Survey  
 $\delta^{15}\text{N}$  data

Multi-element data

Other work

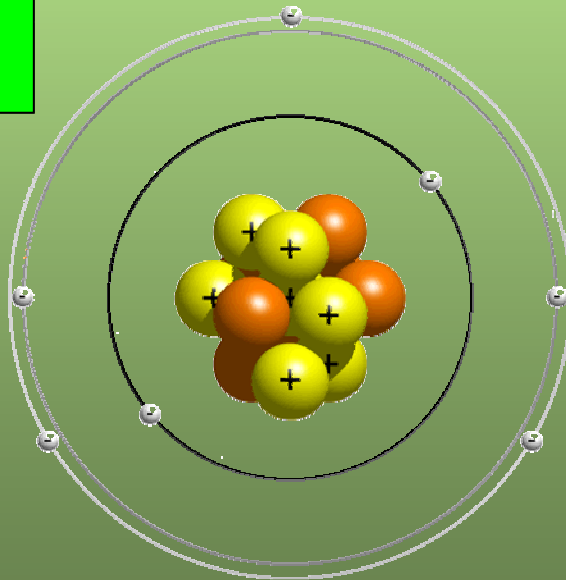
Future work



*'light'* isotope

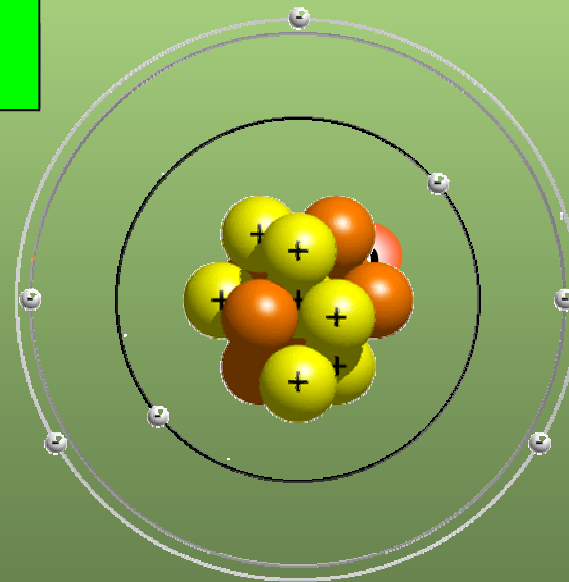
*'heavy'* isotope

**$^{14}\text{N}$   
 $_7$**



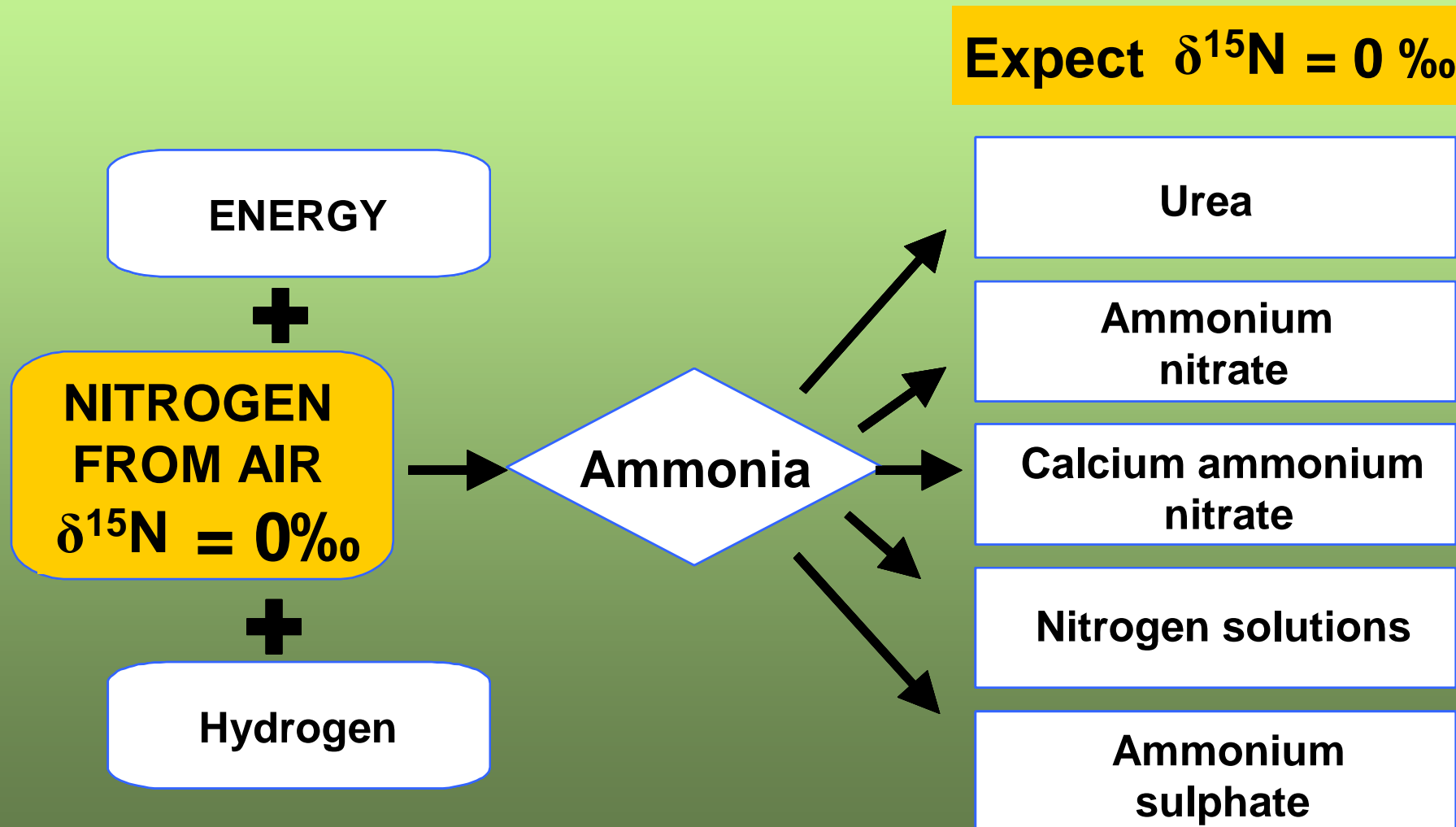
99.63 %

**$^{15}\text{N}$   
 $_7$**



0.37 %

# Chemical fertiliser production



Introduction

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**$^{14}\text{N}$**   
14.00307  
99.63%

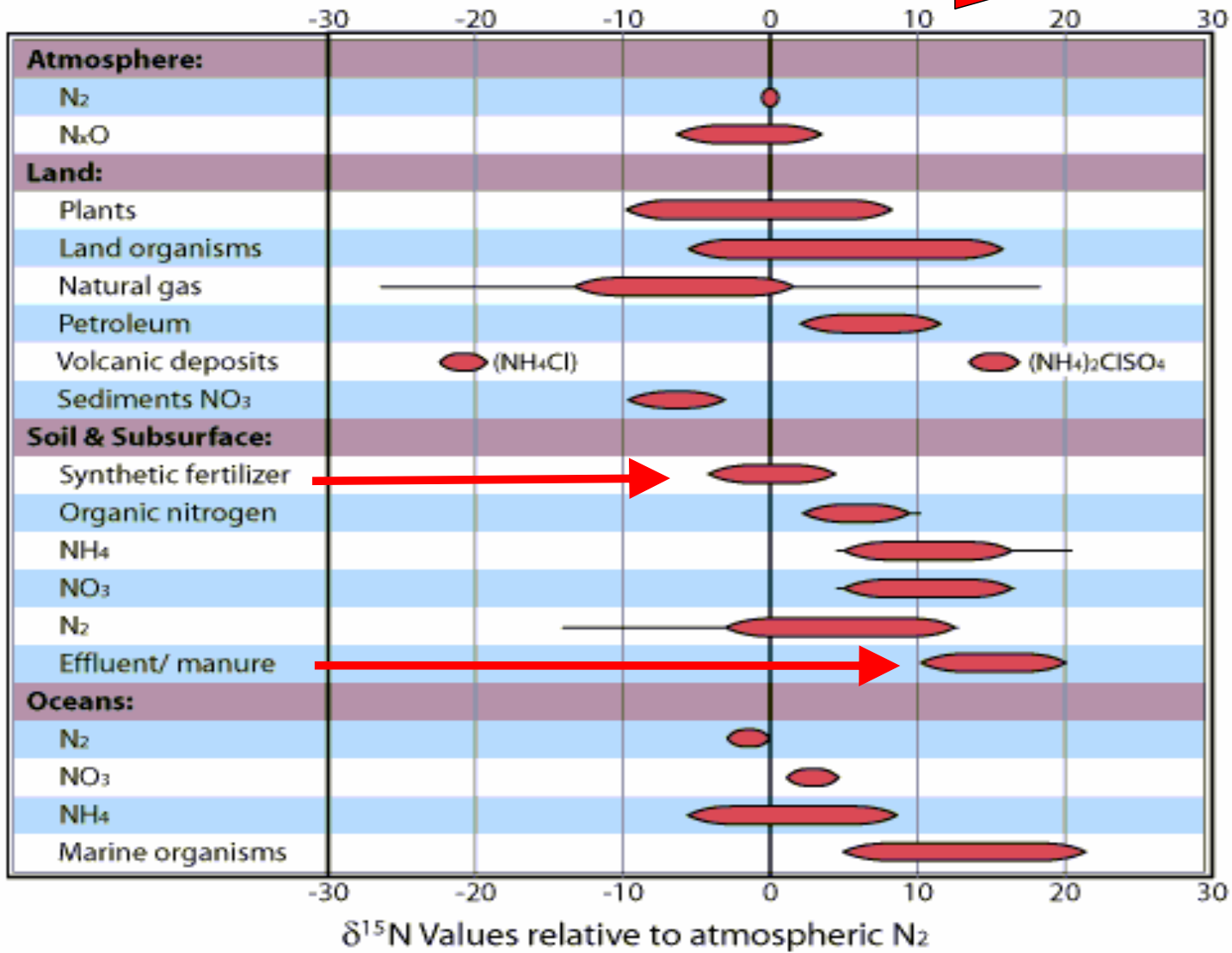
Stable

**$^{15}\text{N}$**   
15.0001  
0.37%

Stable

Increasing  $^{15}\text{N}$  content

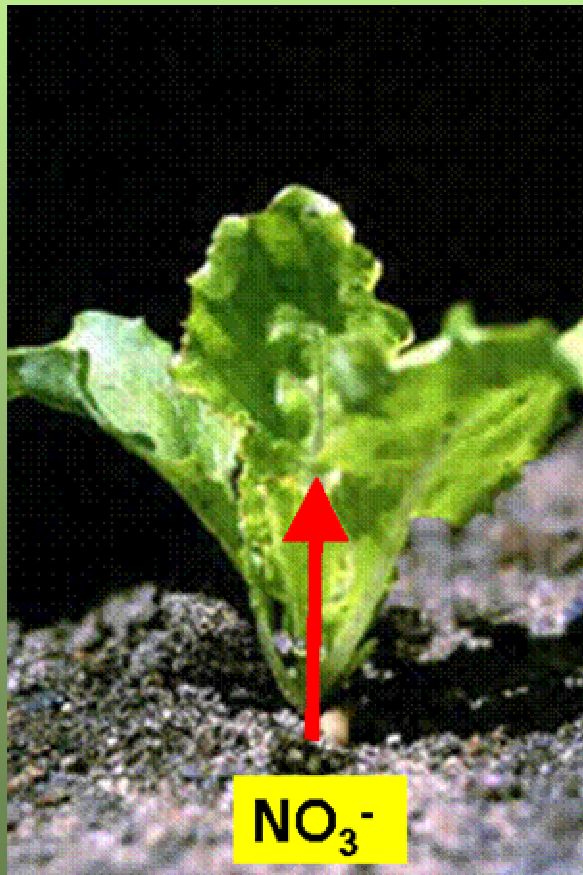
**AS Bateman and SD Kelly (2007)**  
Fertilizer nitrogen isotope signatures,  
*Isotopes in Environmental and Health Studies*, Vol. 43, No. 3, September 2007, 237–247



FROM: SAHRA

<http://www.sahra.arizona.edu/programs/isotopes/nitrogen.html>

# Hypothesis



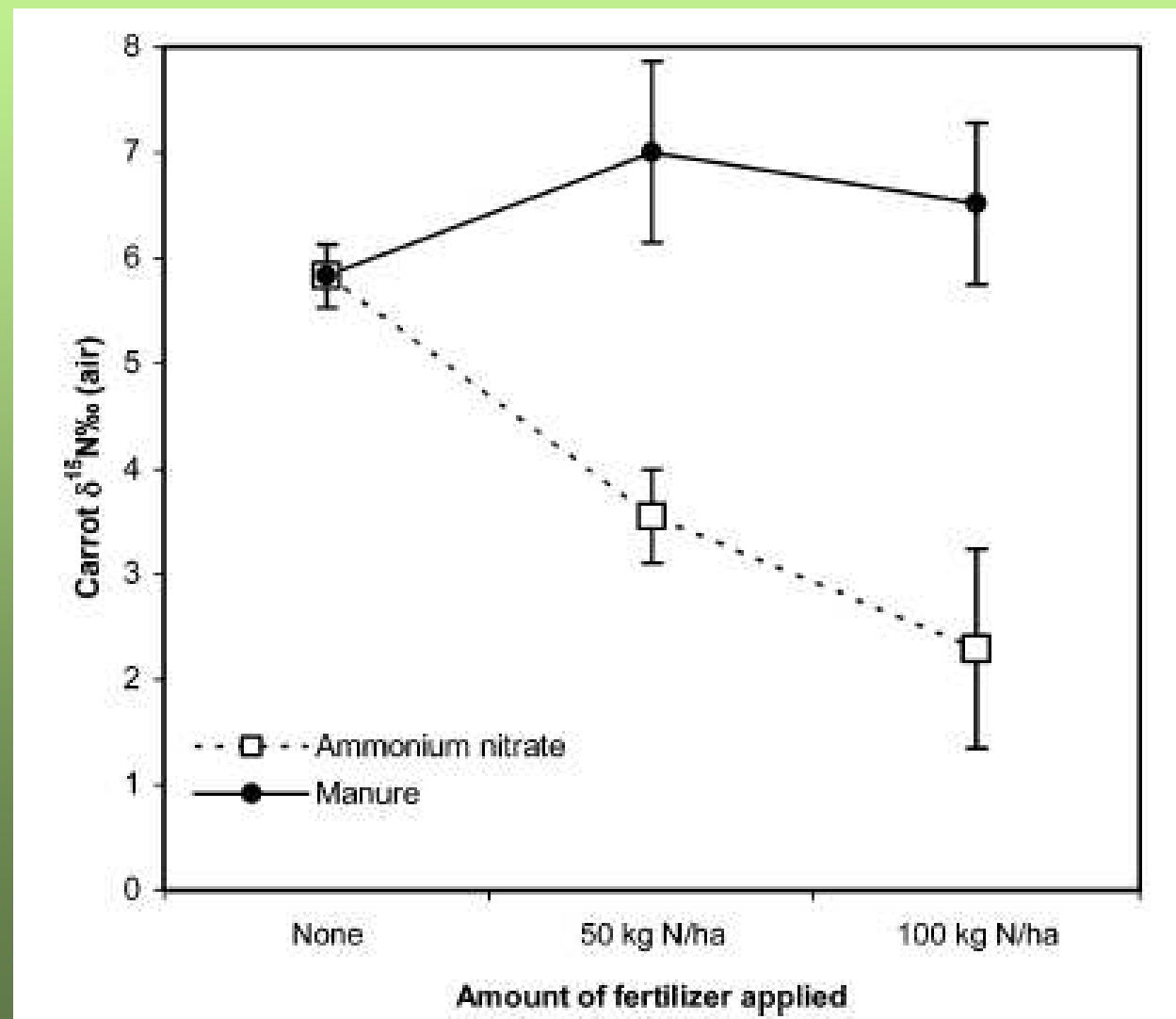
Input of Chemical Fertiliser  
( $\text{NO}_3$  or  $\text{NH}_4$ )

$$-2\text{‰} \leq \delta^{15}\text{N} \leq +2\text{‰}$$

Lower  $\delta^{15}\text{N}$  of available soil  
nitrogen pool

Plants with lower  $\delta^{15}\text{N}$

## Controlled cultivation experiments

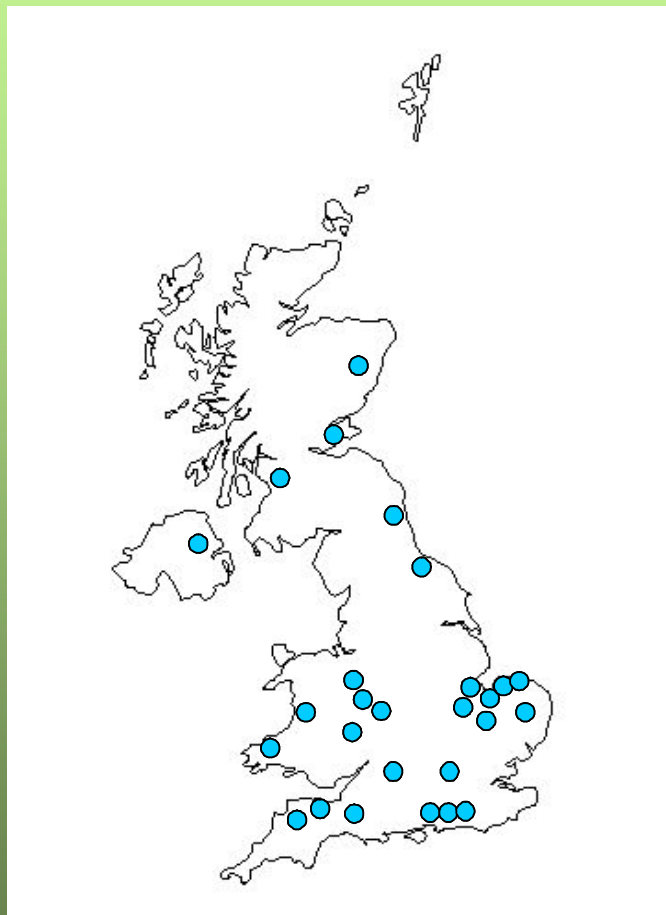


**Carrot**  
**Tomato**  
**Lettuce**  
**Mushroom**

**A S Bateman, S D Kelly & T D Jickells (2005)**

*“Nitrogen Isotope Relationships between Crops and Fertilizer: Implications for Using Nitrogen Isotope Analysis as an Indicator of Agricultural Regime”*  
**Journal of Agricultural and Food Chemistry, 53(14); 5760-5765.**

# Baseline Survey

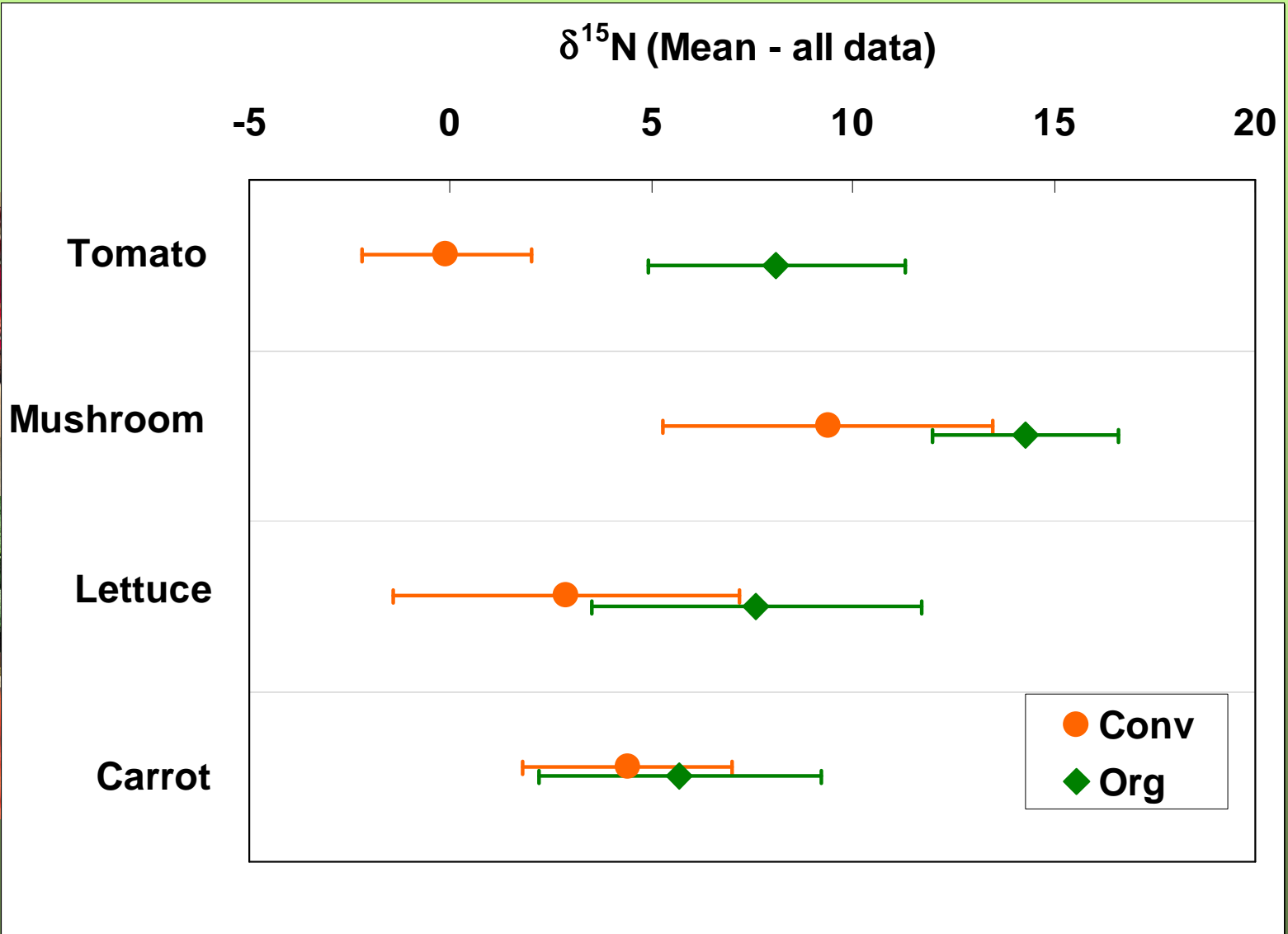


+ some samples from Italy, Germany, Spain & Holland



- Soil type
- Weather
- Time of harvest
- Type and  $\delta^{15}\text{N}$  of synthetic fertiliser
- Type and  $\delta^{15}\text{N}$  of fertiliser applied by organic grower

# Baseline Survey – Summary data



n
43
61
14
11
55
49
18
13

Introduction

Stable isotopes

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Controlled cultivation experiments

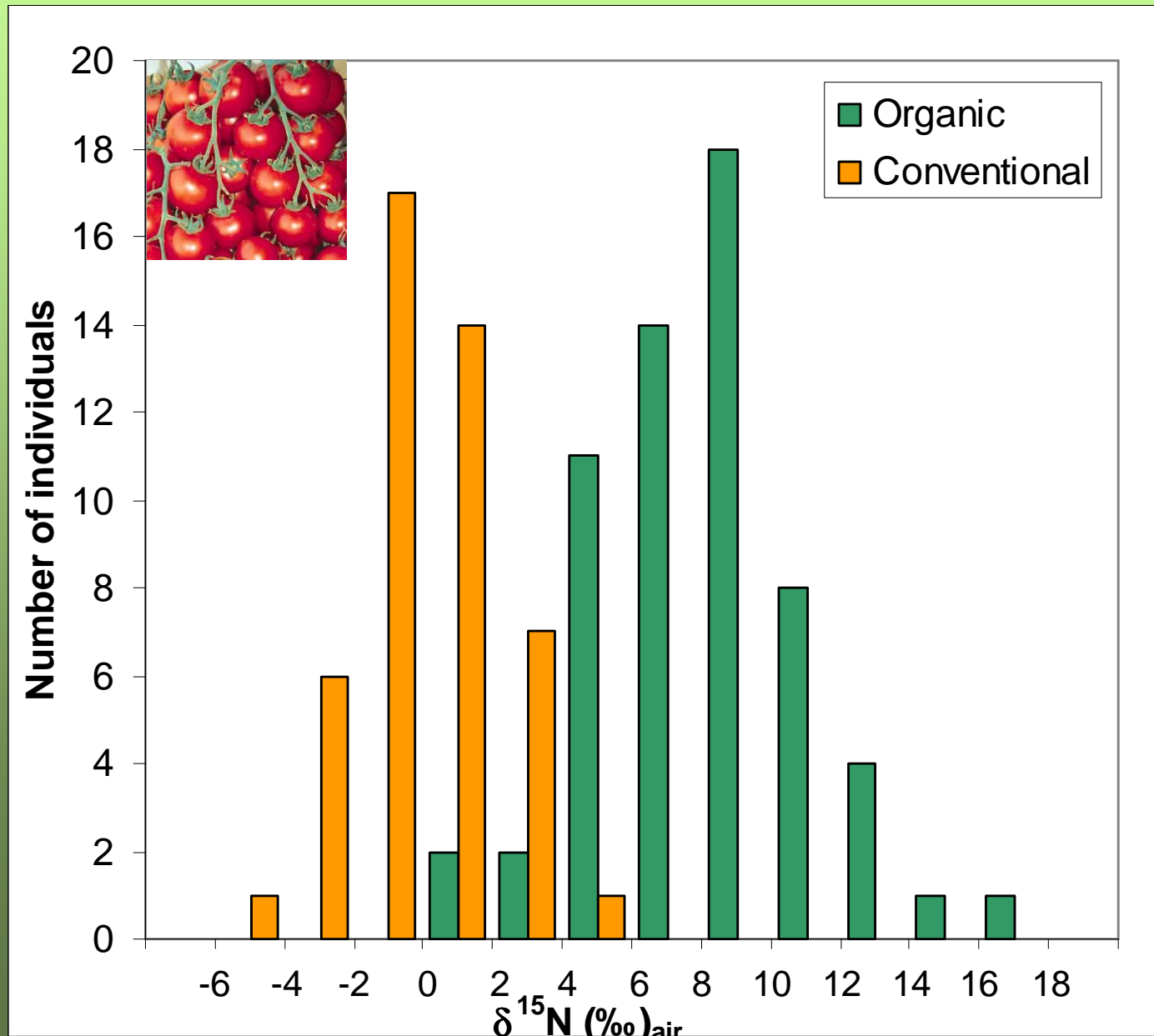
**Baseline Survey**  
 $\delta^{15}\text{N}$  data

Multi-element data

Other work

Future work

# *Tomato data*



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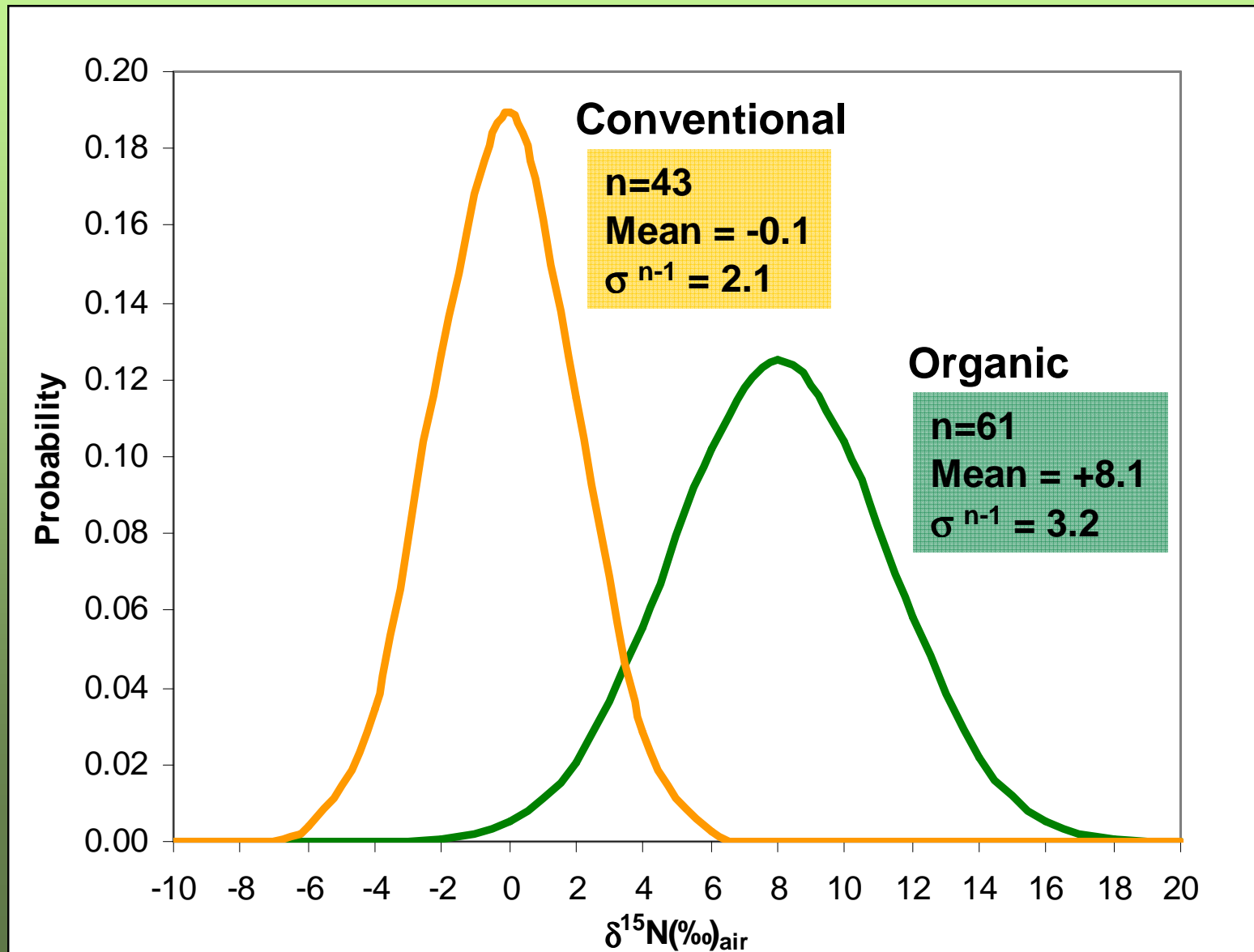
**Baseline Survey**  
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# *Tomatoes – normal dist<sup>n</sup>*



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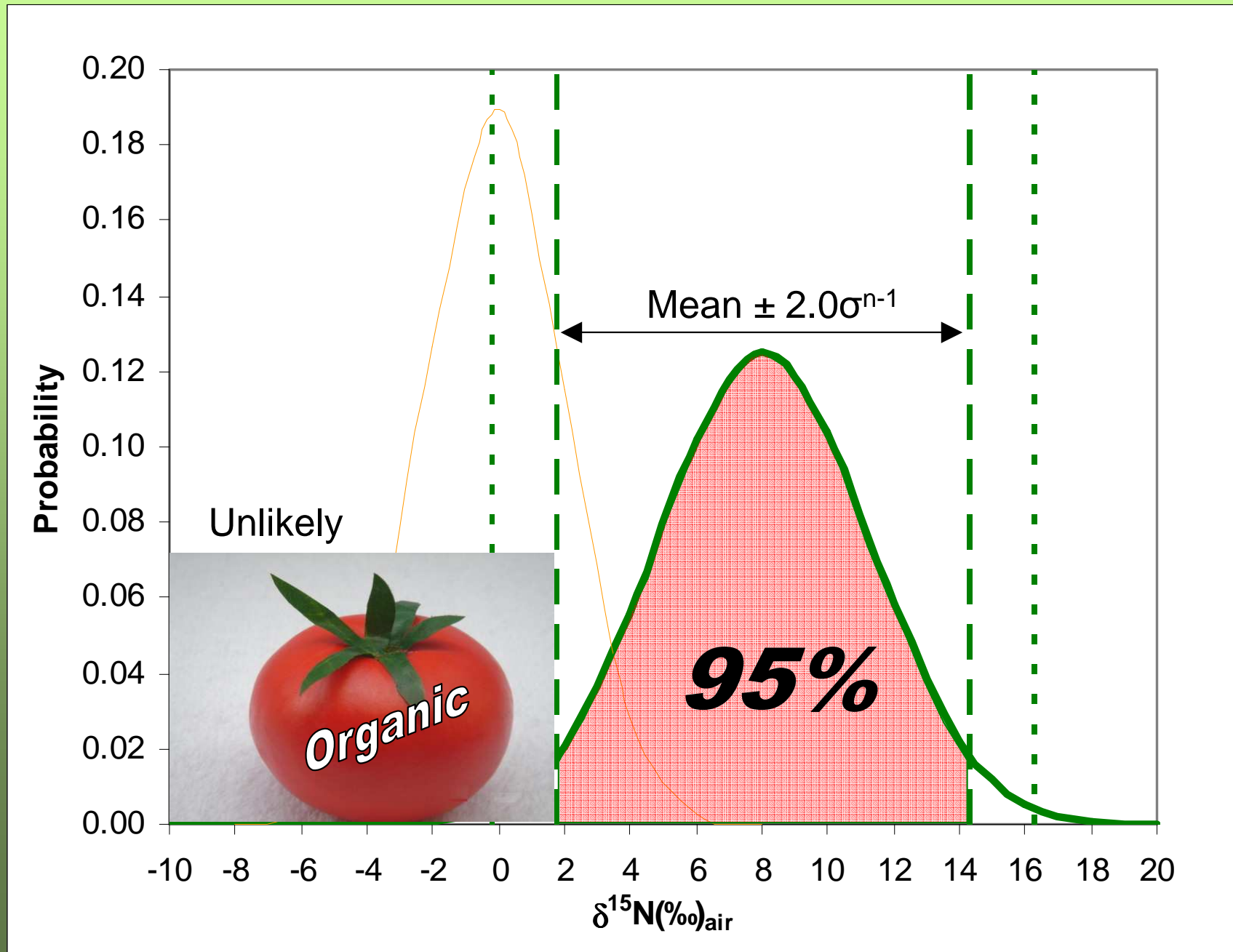
Controlled cultivation experiments

**Baseline Survey**  
 $\delta^{15}\text{N}$  data

Multi-element data

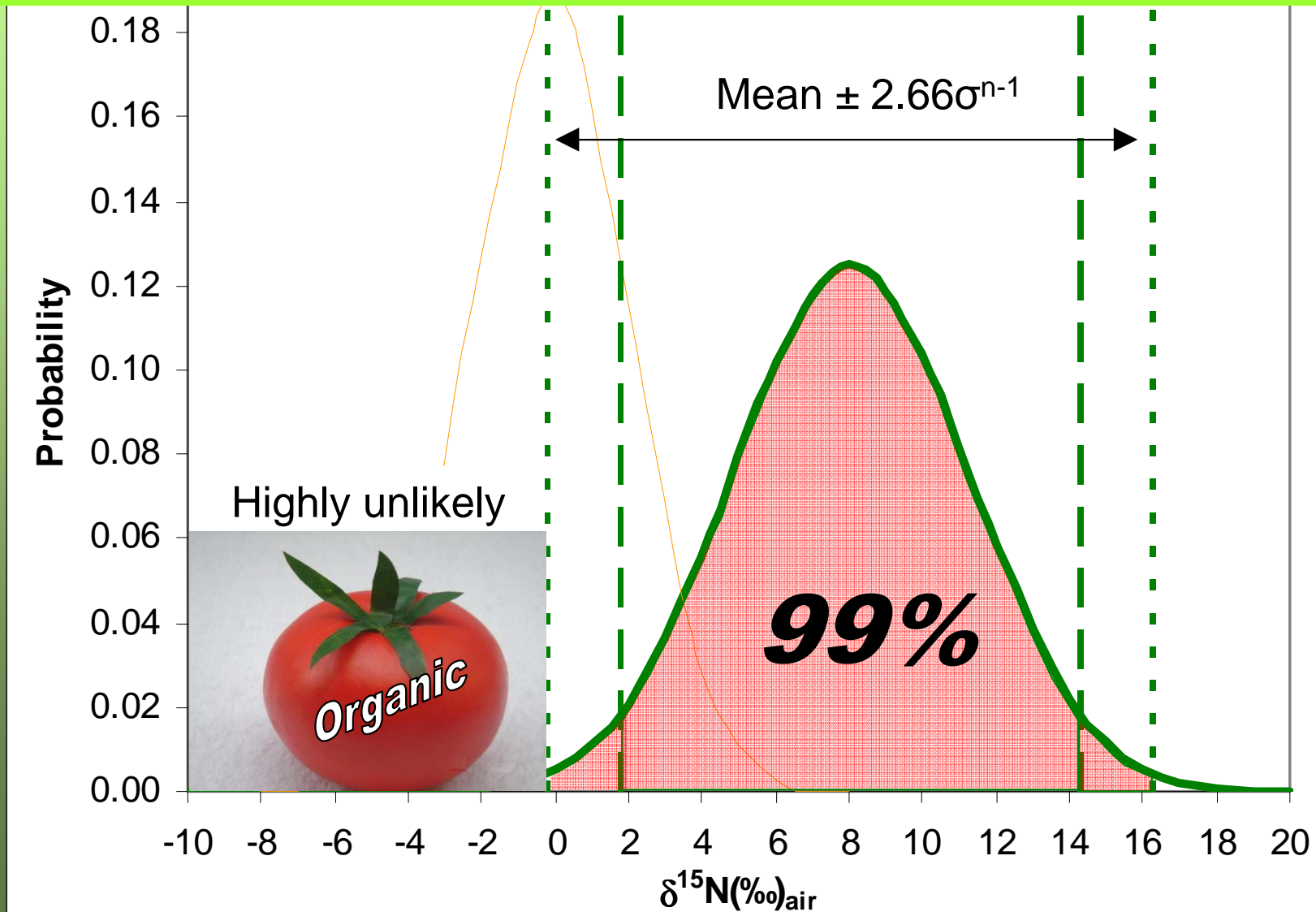
Other work

Future work

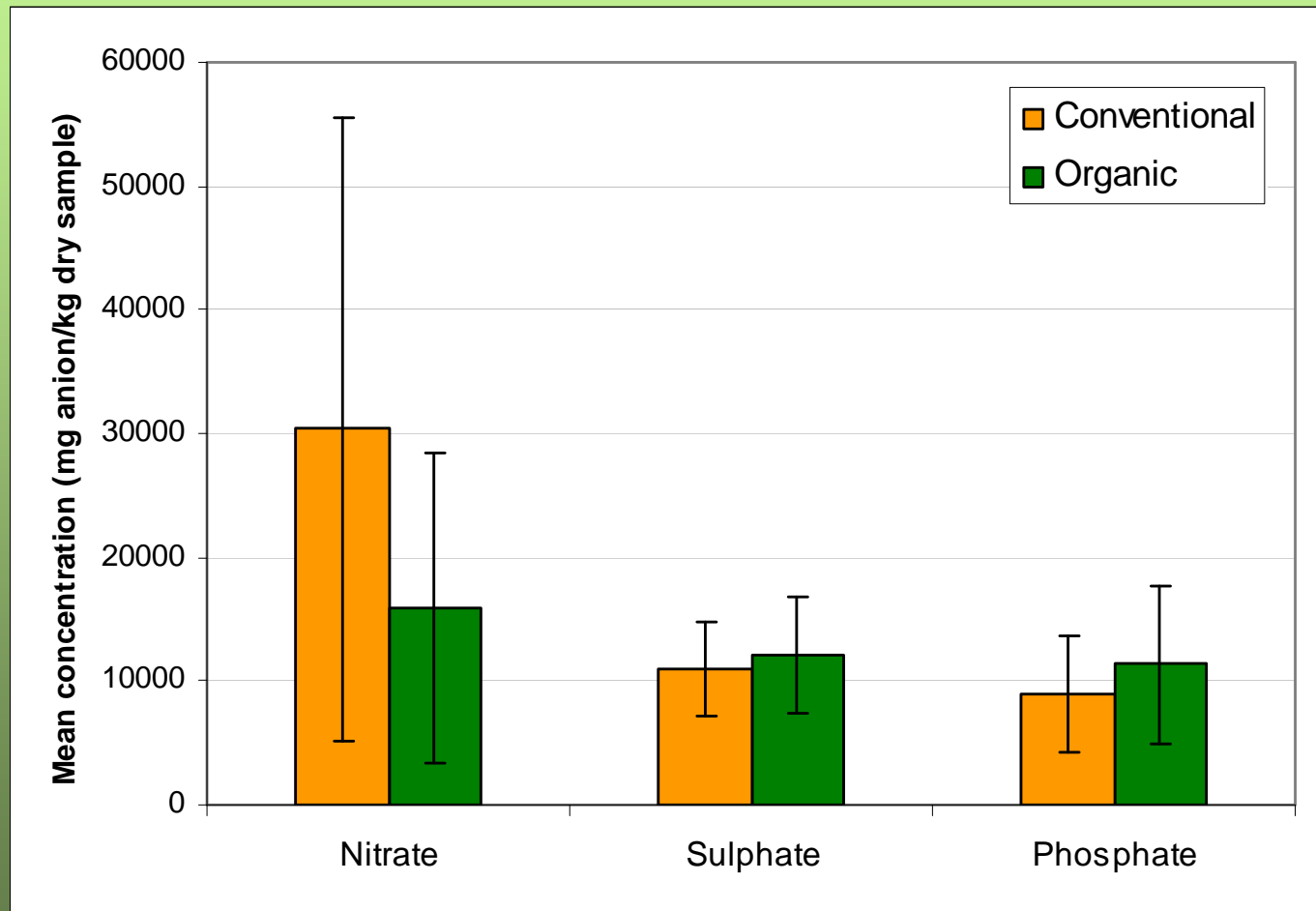


A S Bateman, S D Kelly and M Woolfe (2007)

“Nitrogen isotope composition of organically and conventionally grown crops”  
Journal of Agricultural and Food Chemistry , 55, 2664-2670.



## Major anions – lettuces



- May be related to water content, some evidence that excessive fertiliser application leads to rapid growth at least in part by swelling with more water (Siderer *et al.*, 2005).

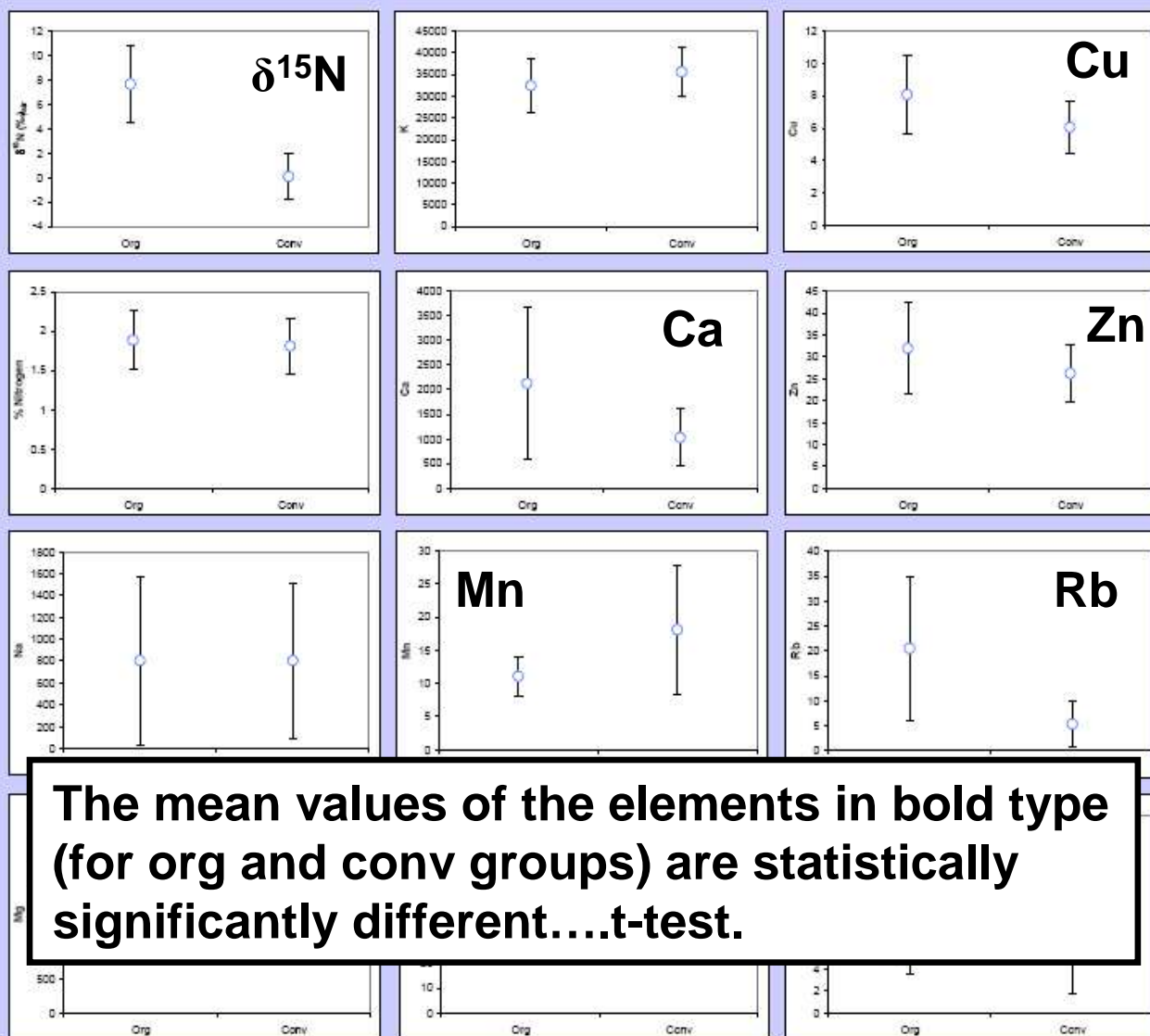
Conv, n=55  
Org, n=48

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## Multi-element signatures

- Measure many elements simultaneously by ICP-MS (Na, Mg, K, Ca, Mn, Fe, Cu, Zn, Rb, Sr, Cd, Ba)
- Identify key variables that separate organic and conventional crops using Canonical Discriminant Analysis (CDA)
- We did not use an '*a priori*' hypothesis so if we see differences, we need to try and explain them.

## Multi-element signatures (tomatoes)



The mean values of the elements in bold type (for org and conv groups) are statistically significantly different....t-test.

**Ca** 2x higher in org  
**Cu + Zn** slightly higher in org  
**Mn** 2x lower in org  
**Rb** 4x higher in org

### Possible explanations

- Cu/Zn used in animal feed supplements, use of manure in org. cultivation?
- Rb usually geographical indicator. Soil grown vs hydroponic?
- More Mn available in hydroponic solution than naturally in soil?
- Mycorrhizal associations?

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**Multi-element data**

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## AMF – Arbuscular Mycorrhizal Fungi



**Mn**



**Rb**



**Ca**



**Zn**



**Cu**



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## Canonical Discriminant Analysis (CDA)

- Looks at differences between two or more groups (org/conv) with respect to many variables (isotope + multi-element data).
- Identifies the variables that are best at placing an unknown sample into its appropriate group.
- Uses a linear combination of the multi-element & isotope data to maximise the separation between the groups.

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## CDA Results

### i) Tomatoes

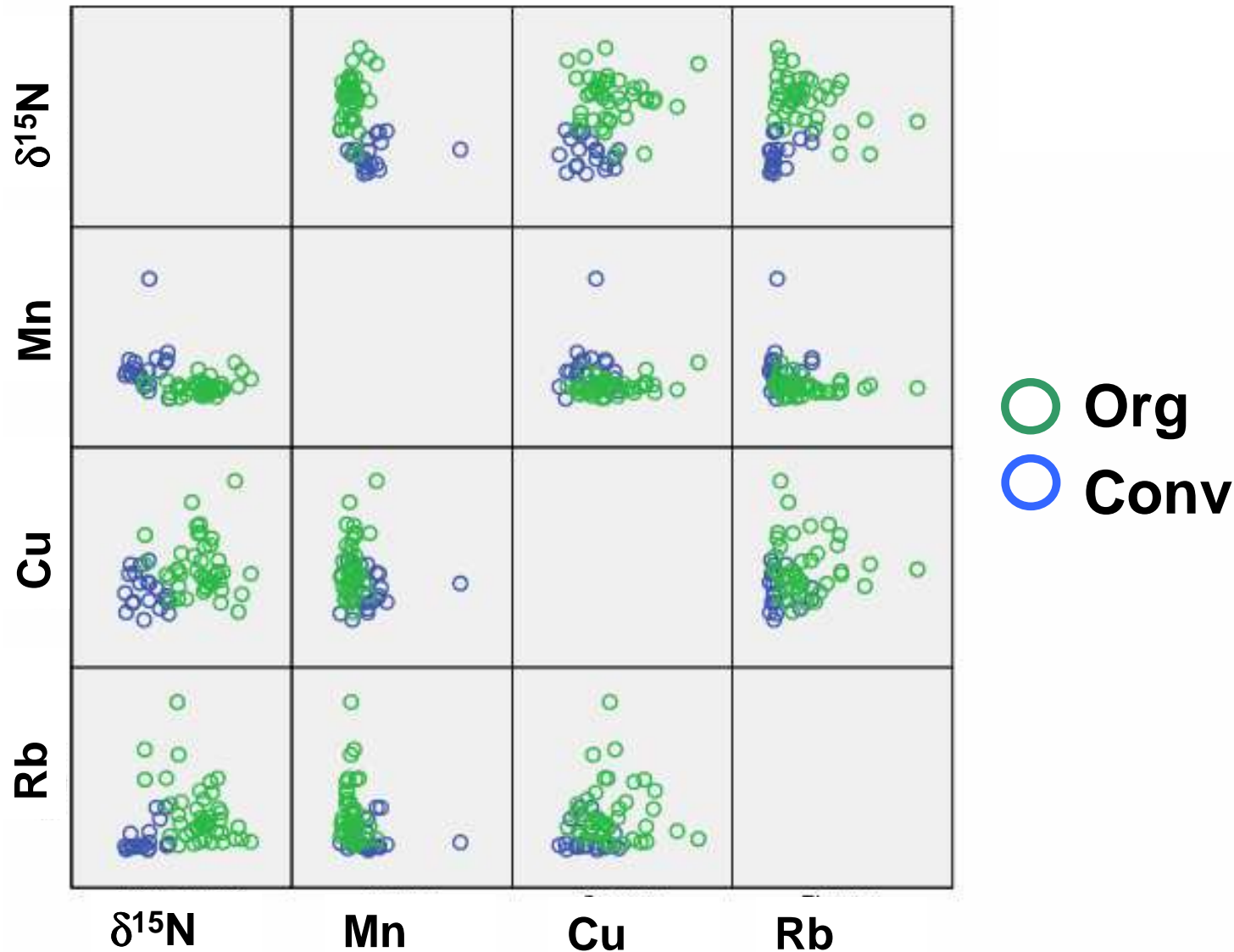
	Variables entered	Predictor variables	Number of individuals	Conventional		Organic		Overall
Year 1	$\delta^{15}\text{N}$ , %N	$\delta^{15}\text{N}$	51	18/18	100%	30/33	90.9%	94.1%
Year 1	$\delta^{15}\text{N}$ , %N, multi-elements	$\delta^{15}\text{N}$ , Rb, Cu, %N	51	18/18	100%	33/33	100%	100%
Year 1 + Year 2	$\delta^{15}\text{N}$ , %N	$\delta^{15}\text{N}$	107	45/46	97.8%	57/61	97.8%	95.3%

### ii) Lettuces

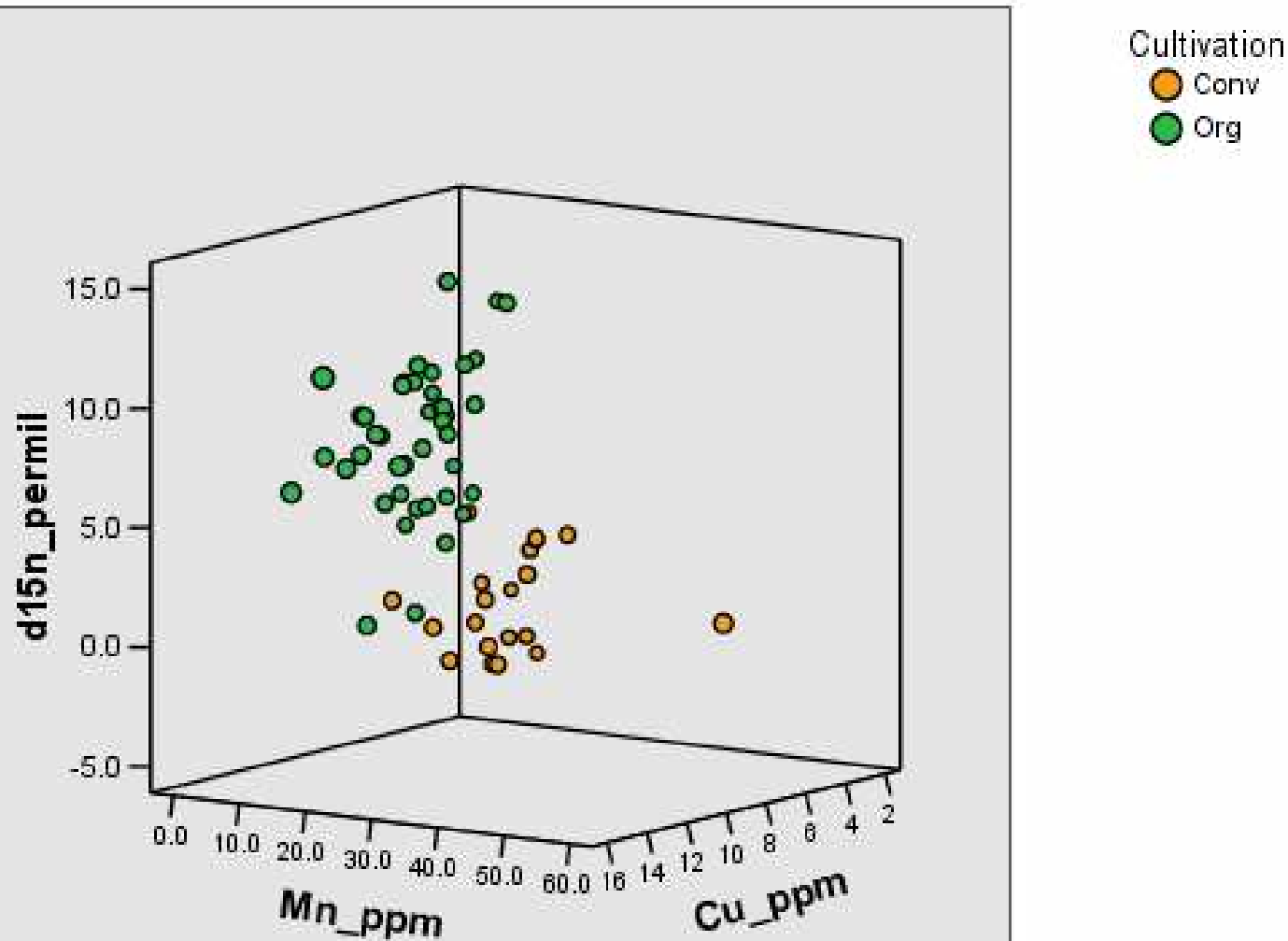
	Variables entered	Predictor variables	Number of individuals	Conventional		Organic		Overall
Year 1	$\delta^{15}\text{N}$ , %N	$\delta^{15}\text{N}$	42	21/28	75%	10/14	71.4%	73.8%
Year 1	$\delta^{15}\text{N}$ , %N, $\text{NO}_3$ , $\text{SO}_4$ , $\text{PO}_4$	$\text{NO}_3$ , $\text{SO}_4$ , $\text{PO}_4$	42	22/28	78.6%	10/14	71.4%	76.2%
Year 1	$\delta^{15}\text{N}$ , %N, $\text{NO}_3$ , $\text{SO}_4$ , $\text{PO}_4$ , multielement data	Rb, $\delta^{15}\text{N}$	42	24/28	85.7%	8/14	57.1%	76.2%
Year 1 + Year 2	$\delta^{15}\text{N}$ , %N	$\delta^{15}\text{N}$	103	46/55	83.6%	30/48	62.5%	73.8%
Year 1 + Year 2	$\delta^{15}\text{N}$ , %N, $\text{NO}_3$ , $\text{SO}_4$ , $\text{PO}_4$	$\delta^{15}\text{N}$ , %N, $\text{NO}_3$ , $\text{SO}_4$	103	45/55	81.8%	34/48	70.8%	76.7%

# arbuscular mycorrhizal fungi (AMF) ?

## Tomatoes



## Tomato Data - 3-D Scatter Plot for variables $\delta^{15}\text{N}\text{‰}$ , Mn (ppm), Cu (ppm) & Rb (ppm)



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# ***Conclusions***

1.  $^{15}\text{N}$  analysis is not definitive but may provide evidence to corroborate whether chemical N fertiliser has been applied to a crop.
2. Methodology is more suitable for some crop types than others e.g. hydroponic conventional versus Organic soil grown tomatoes, peppers..
3. Multi-element analysis improves classification rates from 95% to 100% for tomatoes.
4. Differing concentrations of Mn, Cu and Rb may be attributable to AMF

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# Other work – Patent application

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**(WO/2003/087813) METHOD FOR DISCRIMINATING ORGANIC AGRICULTURAL PRODUCTS FROM CONVENTIONAL AGRICULTURAL PRODUCTS BY USING NITROGEN ISOTOPE INDEX**

Biblio. Data | Description | Claims | National Phase | Notices | Documents

Latest bibliographic data on file with the International Bureau

Publication Number: WO/2003/087813    International Application No.: PCT/KR2003/000740  
 Publication Date: 23.10.2003    International Filing Date: 12.04.2003  
 Chapter 2 Demand Filed: 12.06.2003

Int. Class.: **G01N 33/24** (2006.01)

Applicants: RO, Hee-Myong [KR/KR]; 2-105 Bando Apt., Ichon-1-dong, Yongsan-gu, Seoul 140-031 (KR).  
 CHOI, Woo-Jung [KR/KR]; 408-906 Wooman Jugong Apt., 300, Wooman-dong, Paldal-gu, Suwon-city, Kyonggi-do 442-191 (KR) (US Only).

Inventors: RO, Hee-Myong [KR/KR]; 2-105 Bando Apt., Ichon-1-dong, Yongsan-gu, Seoul 140-031 (KR).  
 CHOI, Woo-Jung [KR/KR]; 408-906 Wooman Jugong Apt., 300, Wooman-dong, Paldal-gu, Suwon-city, Kyonggi-do 442-191 (KR).

Agent: LEE, Young-Pil; The Cheonghwa Building, 1571-18, Seocho-dong Seocho-gu, Seoul 137-874 (KR).

Priority Data: 10-2002-0019956 12.04.2002 KR

Title: METHOD FOR DISCRIMINATING ORGANIC AGRICULTURAL PRODUCTS FROM CONVENTIONAL AGRICULTURAL PRODUCTS BY USING NITROGEN ISOTOPE INDEX

Abstract: The present invention provides a method for discriminating an organic farmland from a conventional farmland, comprising: (a) measuring an amount of nitrogen isotope in a soil sample of a farmland; (b) calculating a nitrogen isotope index according to the formula (I) using the resultant nitrogen isotope value and (c) determining whether the farmland belongs to an organic farmland or a conventional farmland. According to the present invention, it is possible to objectively discriminate an

Fertilizer Type	Nitrogen Isotope Index (‰)
ENGRAIS CHIMIQUE	~ -2.5
COMPOST ORGANIQUE	~ 17.5

*Patents applied for in...*

Europe

United States

Mexico

China

Canada

Australia

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**Other work**

Future work

# Other work

Accred Qual Assur (2002) 7:378–387  
DOI 10.1007/s00769-002-0531-6

GENERAL PAPER

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Elisabete A. De Nadai Fernandes  
Fábio S. Tagliaferro  
Adriano Azevedo-Filho  
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THE USE OF NITROGEN ISOTOPIC ANALYSIS TO DISTINGUISH BETWEEN ORGANIC AND CONVENTIONAL PRODUCTS

Eric Jamin\*, Stéphanie Lescuyer, Delphine Salvat-Brunaud, Michèle Lees

*Eurofins Scientific Analytics, Nantes, France* \*Corresponding author e-mail :  
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**Application of Nitrogen Stable Isotope Ratio as an Indicator for Organic Fertilized Vegetable**

*Akimasa NAKANO and Yoichi UEHARA*

**Bulletin of the National Institute of Vegetable and Tea Science, 3, 119-128**

JESIUM 2004

A Stable Future Posters

**Isotope characteristics of vegetables and wheat from conventional and organic production**

JESIUM, 2004, Vienna

A Stable Future

id, Andreas Rossman, Susanne  
rmann Schnitzler, Michael Georgi,  
Gerhard Zimmermann and Ralf  
Winkler

**Organic vegetables: Authentication using stable isotopes**

Rogers, Karyn

Rafter Isotope Laboratory, Geological  
31-312, Lower Hutt, New Zealand, I

SHORT COMMUNICATIONS

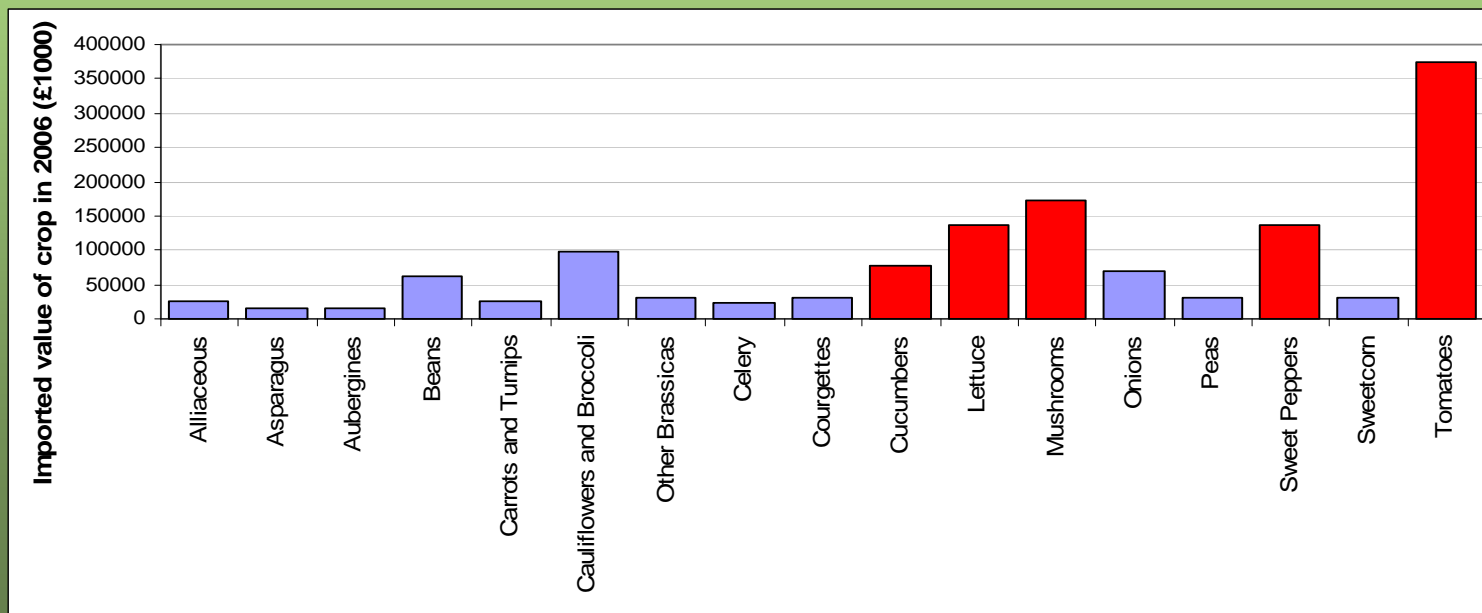
Published in J. Environ. Qual. 37:182–185 (2008).  
doi:10.2134/jeq2007.0329

**Isotopic Discrimination as a Tool for Organic Farming Certification in Sweet Pepper**

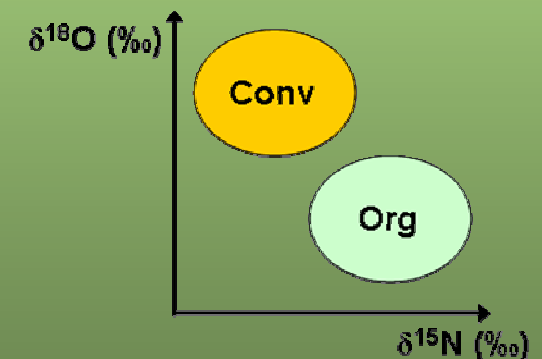
Francisco M. del Amor\* and Joaquín Navarro Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario  
Pedro M. Aparicio UPNA CSIC

## Future Work - we would like to...

- Extend the existing databases to include more EU (non-UK) and worldwide samples
- Target other high value horticultural crops and extend databases to see if exploitable differences in the  $\delta^{15}\text{N}$  of org/conv crops exist, e.g. sweet pepper and cucumbers.
- Analyse the  $\delta^{18}\text{O}$  of extracted nitrate from organic/conventional crops to see if differences exist
- Examine the applicability of secondary metabolite profiling (metabolomics)



### Dual isotope approach



# Acknowledgements

*IFR co-workers : D Hart, H Ueckermann & J Hoogewerff  
for TE measurements*



*For funding this research*

Thank you for your attention!

Simon.Kelly@bbsrc.ac.uk