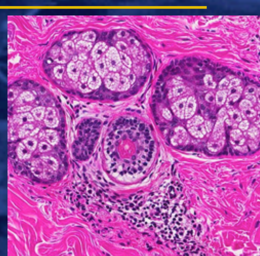
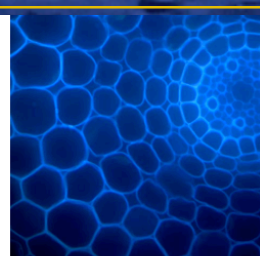
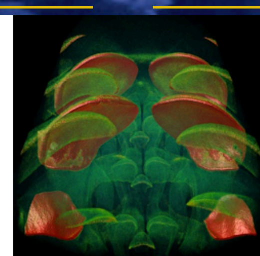
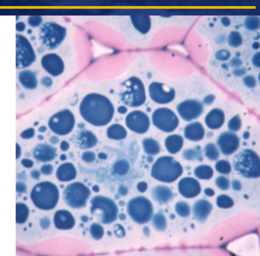
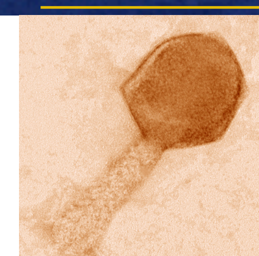
THE UNIVERSITY OF WESTERN AUSTRALIA

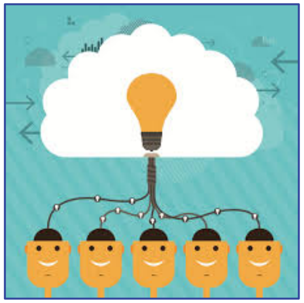
To the rhizosphere & beyond: the past, the present, & future opportunities

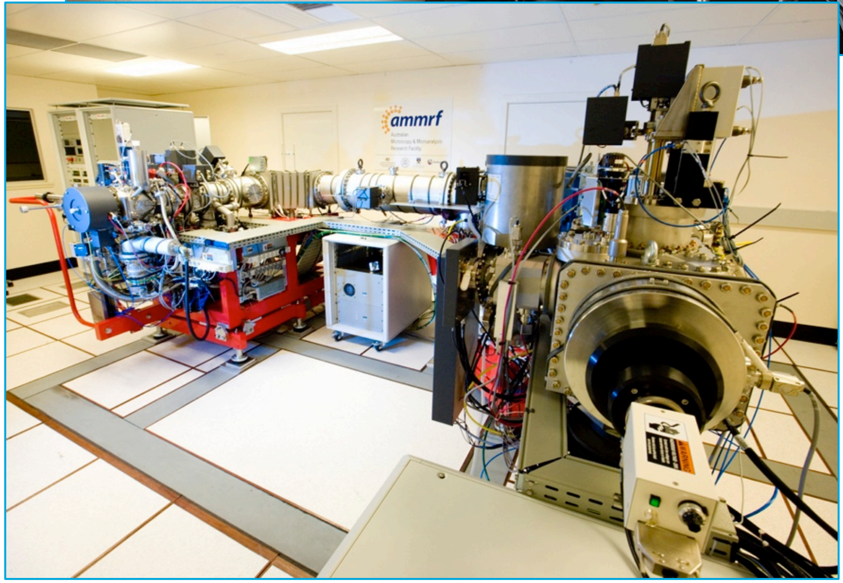
Associate Professor Peta Clode

Centre for Microscopy, Characterisation & Analysis

CMCA @ UWA

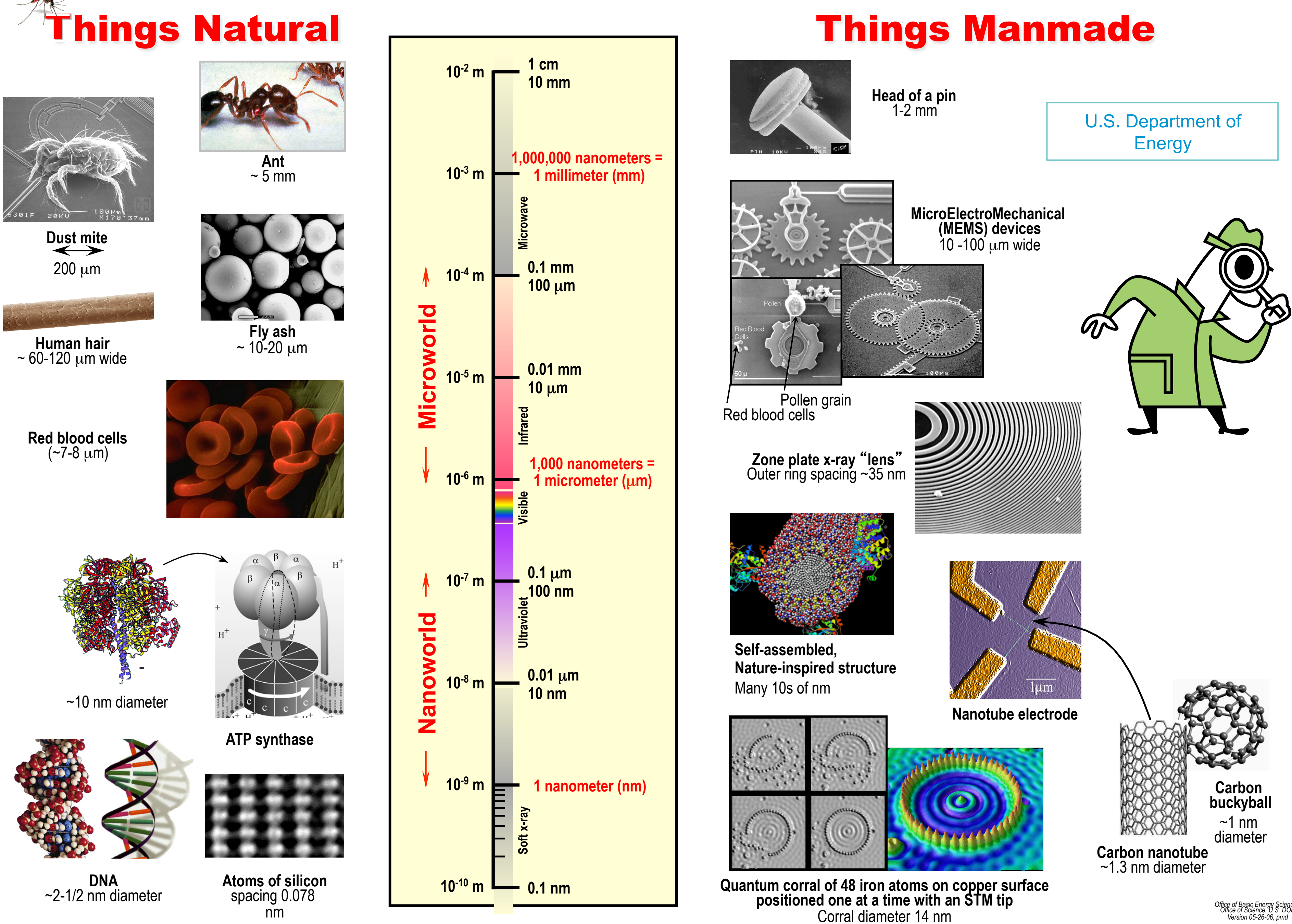


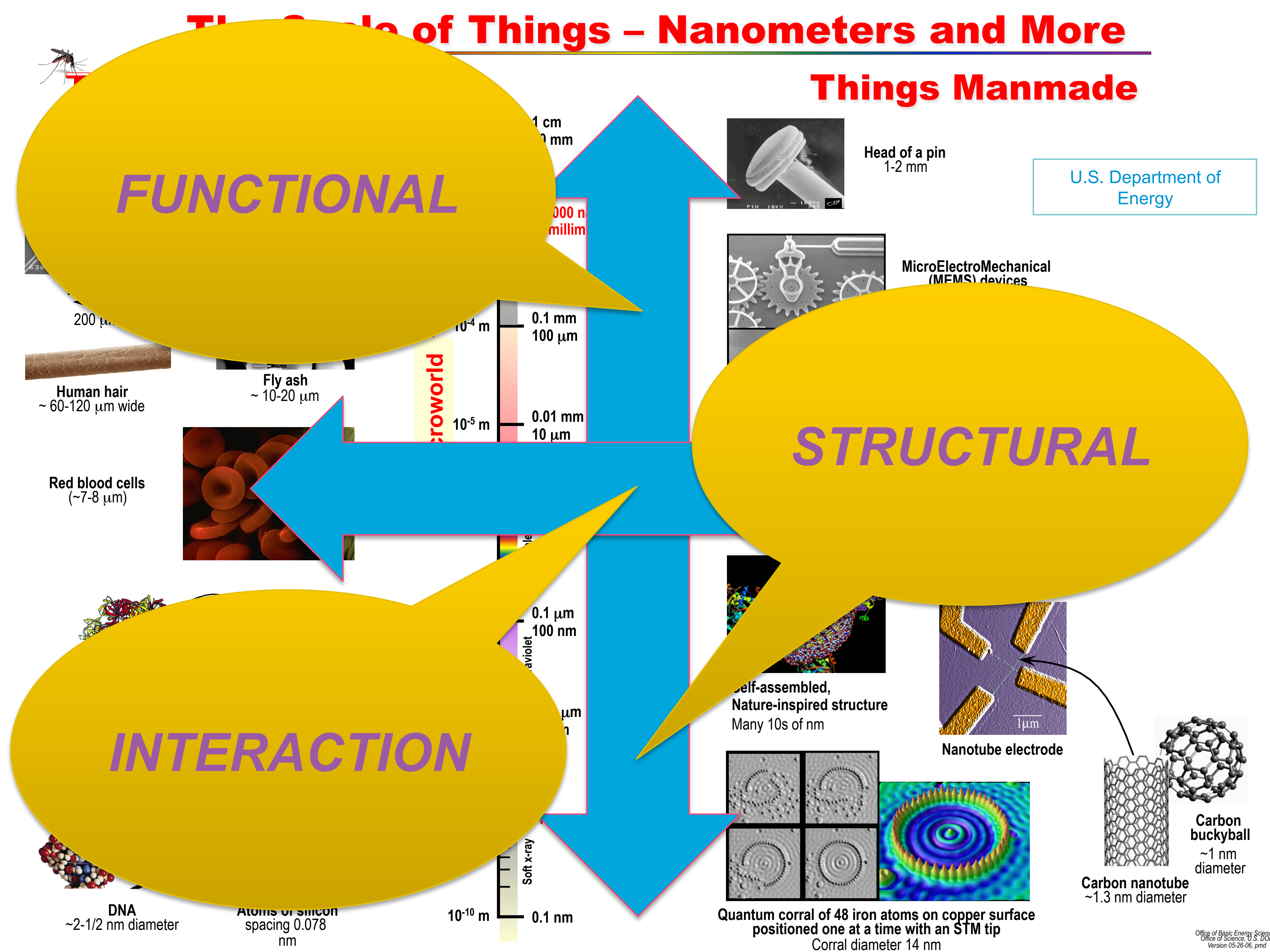


Facilities

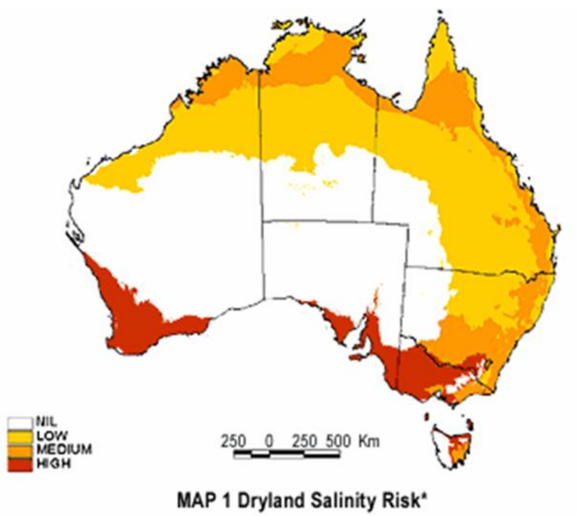
- AMMRF Flagship Ion Probe Facility
- Metabolomics Australia
- NIF Bioimaging
- Cytometry
- Biological and molecular mass spectrometry (BMMS)
- X-ray microscopy (XRM)
- Nuclear magnetic resonance
- Optical microscopy
- Scanning electron microscopy (SEM)
- Scanning probe microscopy (SPM)
- Transmission electron microscopy (TEM)
- X-ray diffraction
- Data management, analysis and visualisation (DMAV)
- Sample preparation
- Physical Containment Level 2

The Scale of Things – Nanometers and More




of Things – Nanometers and More
Things Manmade
U.S. Department of Energy
FUNCTIONAL
STRUCTURAL
INTERACTION
Head of a pin
1-2 mm
MicroElectroMechanical
1 cm
0.1 mm
100 µm
10^{-4} m
0.01 mm
10 µm
10^{-5} m
Human hair
~ 60-120 µm wide
Fly ash
~ 10-20 µm
Red blood cells
(~7-8 µm)
0.1 µm
100 nm
Nature-inspired structure
Many 10s of nm
Nanotube electrode
1µm
Carbon buckyball
~1 nm diameter
Carbon nanotube
~1.3 nm diameter
Quantum corral of 48 iron atoms on copper surface
positioned one at a time with an STM tip
Corral diameter 14 nm
DNA
~2-1/2 nm diameter
spacing 0.078 nm
10^{-10} m
0.1 nm
Soft x-ray
Version 05-26-06, pmd

One handful of a fertile soil can contain more organisms than people on earth (6.7 billion)



>25,000 km's of coastline

Soil Biology / Science

THE UNIVERSITY OF WESTERN AUSTRALIA

- Food security
- Optimised land use
- Biodiversity hotspots
- Ecosystem rehabilitation

GRDC
Grains Research & Development Corporation

Soil Biology / Science

- Food security
- Optimised land use
- Biodiversity hotspots
- Ecosystem rehabilitation

Interactions & activity between plants, soil, additives (*e.g.* biochar), and microorganisms –
WHO's DOING WHAT, WHEN & HOW...?

NanoSIMS & Soil Biology

NanoSIMS

- Dry & stable
- Conductive
- Ultra high vacuum tolerant (10^{-10} torr)
- Very flat

Soil interface

- Soil physics:
  Intact soil structure
- Soil chemistry:
  No modification of N pools
- Soil biology:
  No modification of soil microbial or plant root cells

This poses some difficult challenges

and compromises…

Proof of Concept - 2005

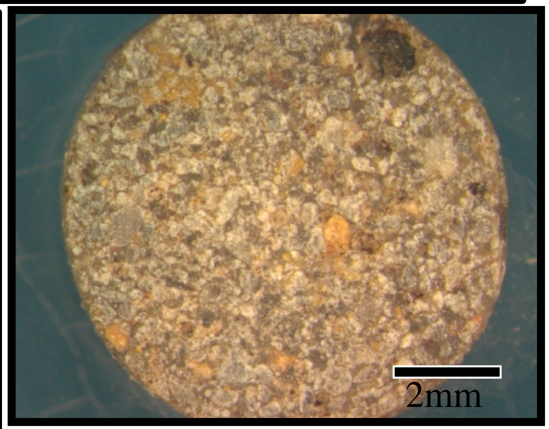
THE UNIVERSITY OF WESTERN AUSTRALIA

??? Can we *prepare & image single ^{15}N-labelled bacteria in a soil core* using NanoSIMS…?

Artificial experiment

THE UNIVERSITY OF WESTERN AUSTRALIA

- *Pseudomonas fluorescens* grown in mineral medium with ^{15}N (54 atom%)
- Bacterial cells added to soil
- Soil fixed and resin embedded
- =10 mm diameter soil cores
- Au coated
- 10-30 micron fields of view
- Simultaneous isotope detection
  - ^{15}N (As ^{12}C^{15}N)
  - ^{14}N (As ^{12}C^{14}N)
  - ^{12}C
  - ^{28}Si



RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2007; 21: 29–34
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.2811
RCM

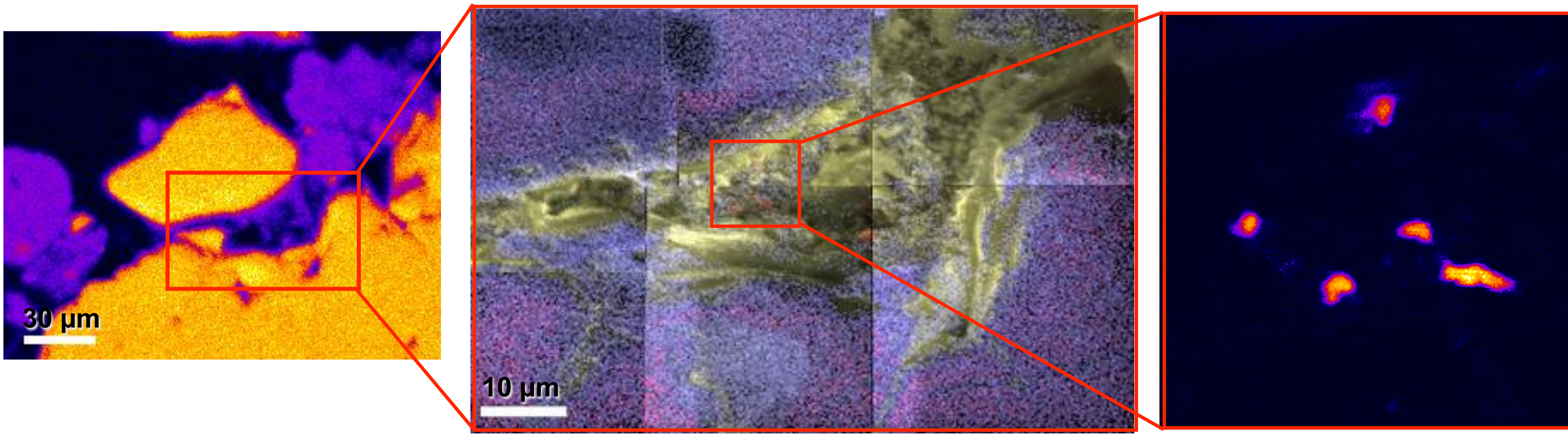
A novel method for the study of the biophysical interface in soils using nano-scale secondary ion mass spectrometry

Anke M. Herrmann[1,5*,†], Peta L. Clode[2], Ian R. Fletcher[2], Naoise Nunan[3], Elizabeth A. Stockdale[4], Anthony G. O'Donnell[5] and Daniel V. Murphy[1]

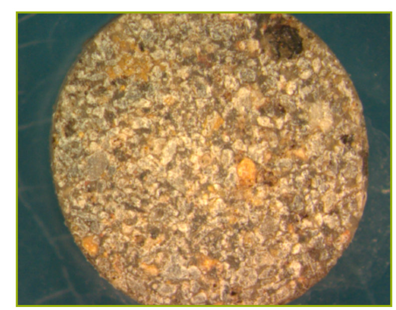
Proof of Concept - 2005

THE UNIVERSITY OF WESTERN AUSTRALIA

Scanning Electron Microscope (SEM)	NanoSIMS Silica	NanoSIMS $^{15}N/^{14}N$ showing ^{15}N labelled bacteria



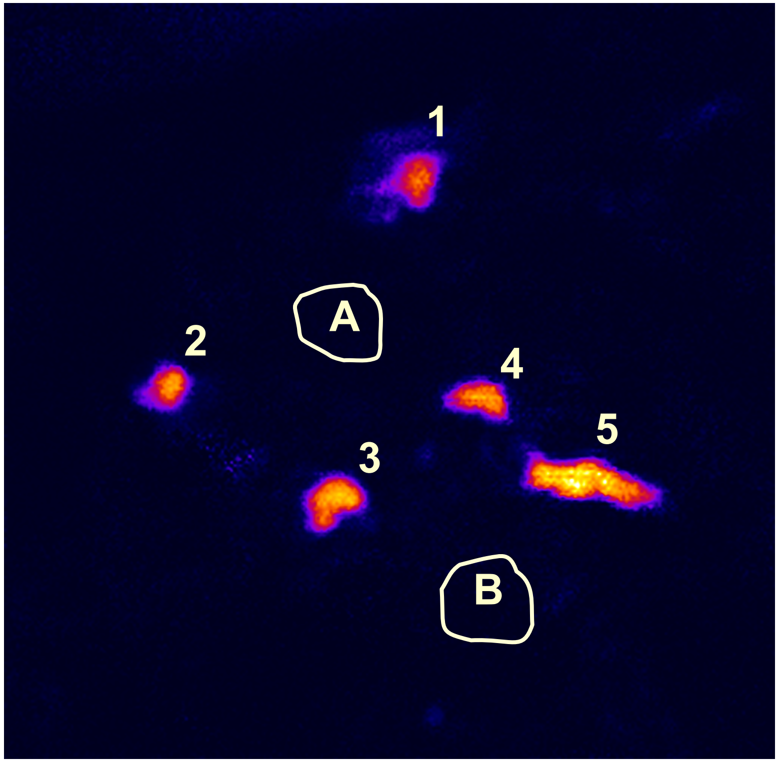
185 x 140 µm FOV X-ray analysis of silica	75 x 50 µm FOV (6 separate images)	10 x 10 µm FOV



Herrmann et al (2007) *Rapid Communications in Mass Spectrometry* **21**, 29-34

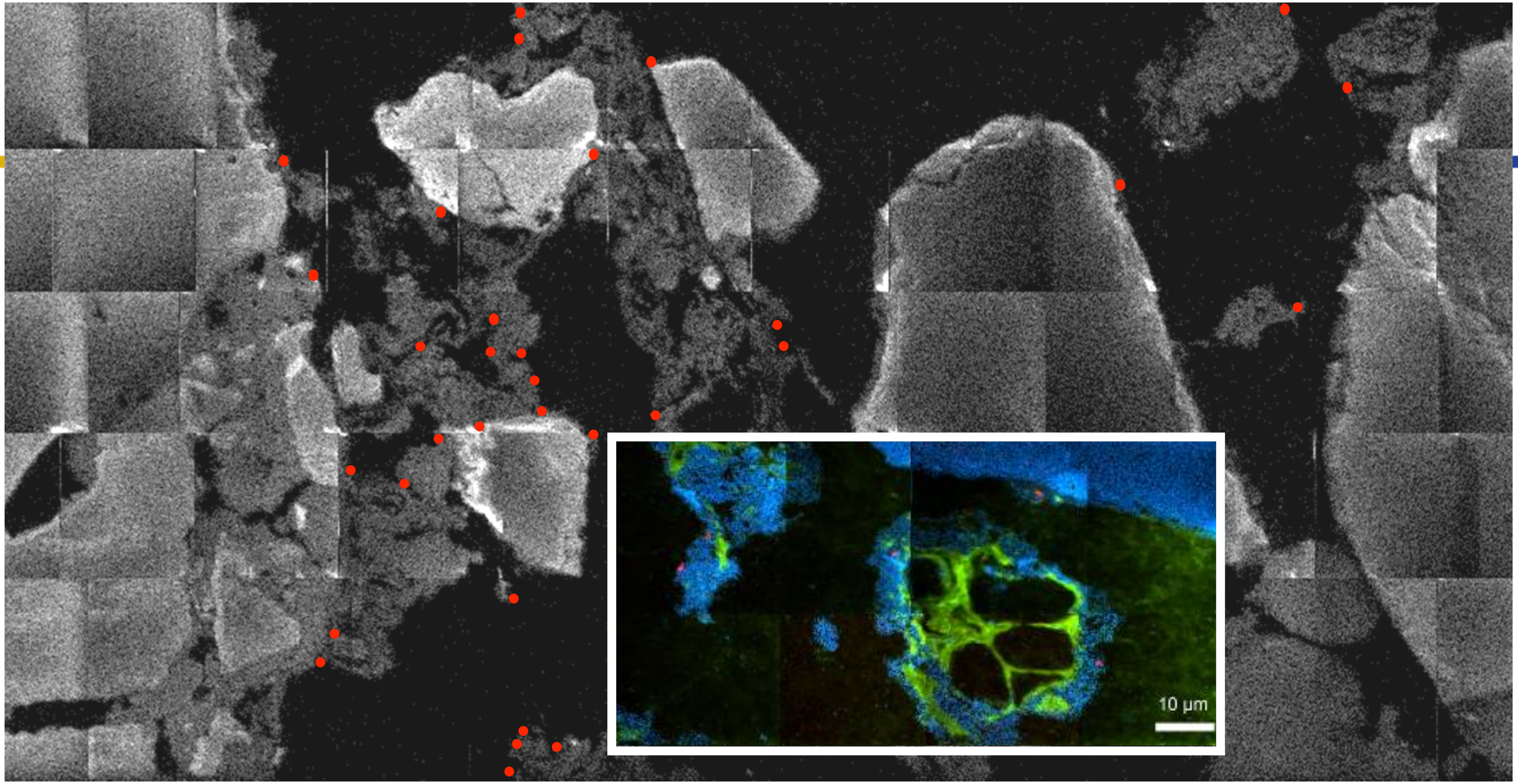
Mapping location of active microbial populations

Can statistically analyse
& compare levels of ^{15}N enrichment in
single cells



Area	$^{15/14}N$ ratio of bacteria
1	0.424 ±0.002
2	0.486 ±0.003
3	0.548 ±0.006
4	0.543 ±0.018
5	0.632 ±0.017
A_{resin}	0.006 ±0.001
B_{resin}	0.009 ±0.001
Natural	0.003

Herrmann et al (2007) *Rapid Communications in Mass Spectrometry* **21**, 29-34



Blue = $^{28}Si^{-}$ Green = $^{12}C^{14}N^{-}$ (represents organic matter)

Red = $^{15/14}N$ ratio images (distribution ^{15}N enriched bacteria)

Herrmann et al (2007) *Rapid Communications in Mass Spectrometry* **21**, 29-34

Stage 2 - 2007

THE UNIVERSITY OF WESTERN AUSTRALIA

Proof of concept

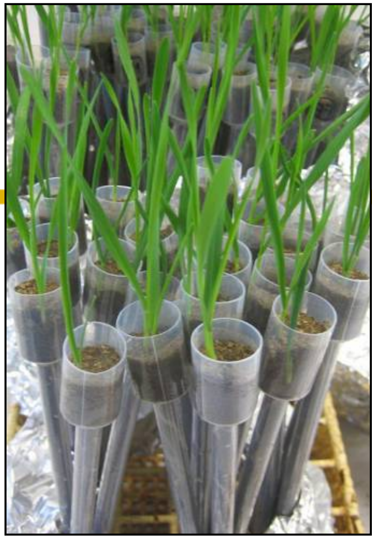
✓ Can we prepare & find ^{15}N-labelled bacteria in a soil core?

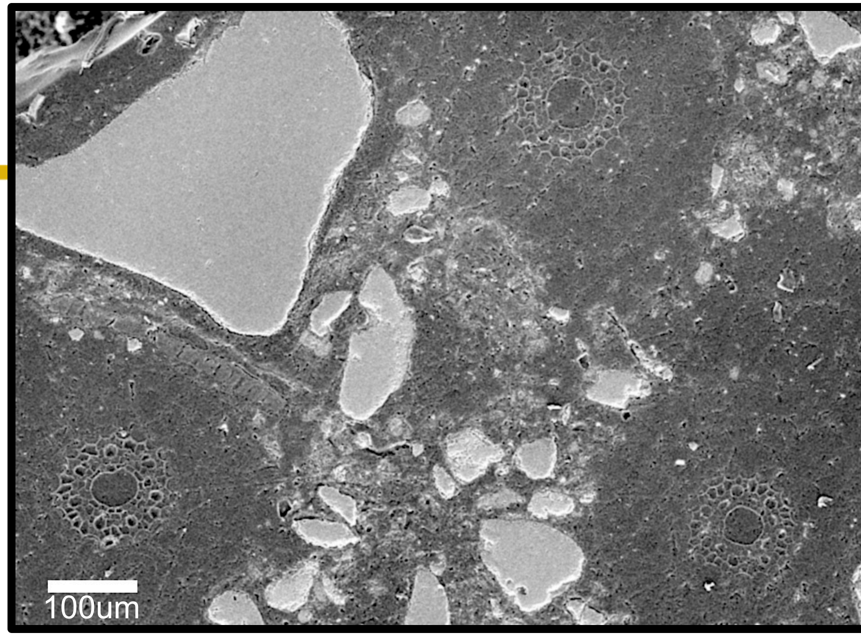
Real experiments & real samples

??? Can we *analyse and track ^{15}N competition between bacteria and plant roots in the rhizosphere in soil*…?

Eg. 1: $^{15}NH_4$ uptake in wheat roots

- Plant grown in soil in a rhizo-tube
- ^{15}N labeled ammonium added at time = 0 hrs
- Plant roots and soil chemically fixed after 5 min, 30 min, 90 min, 6 hr and 24 hr
- Soil resin embedded and polished



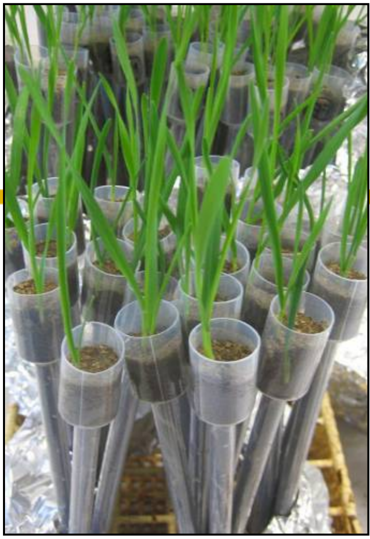


Clode et al. (2009) *Plant Physiology* 151:1751-1757.

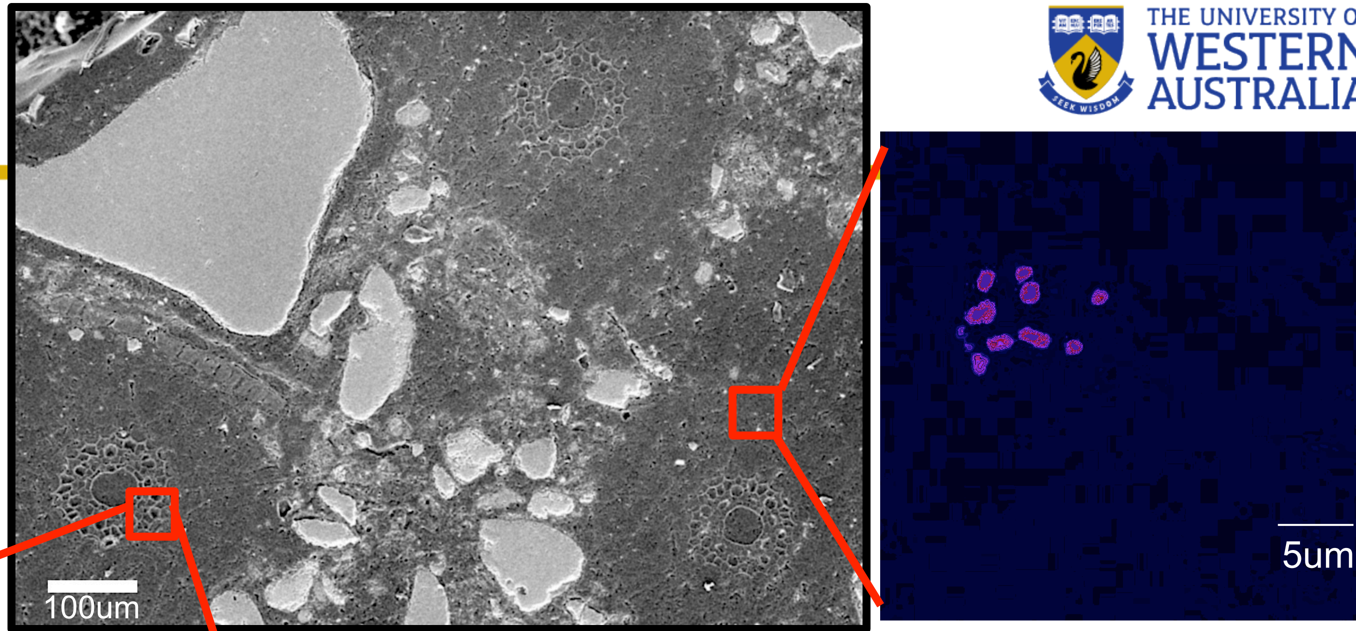
Breakthrough Technologies

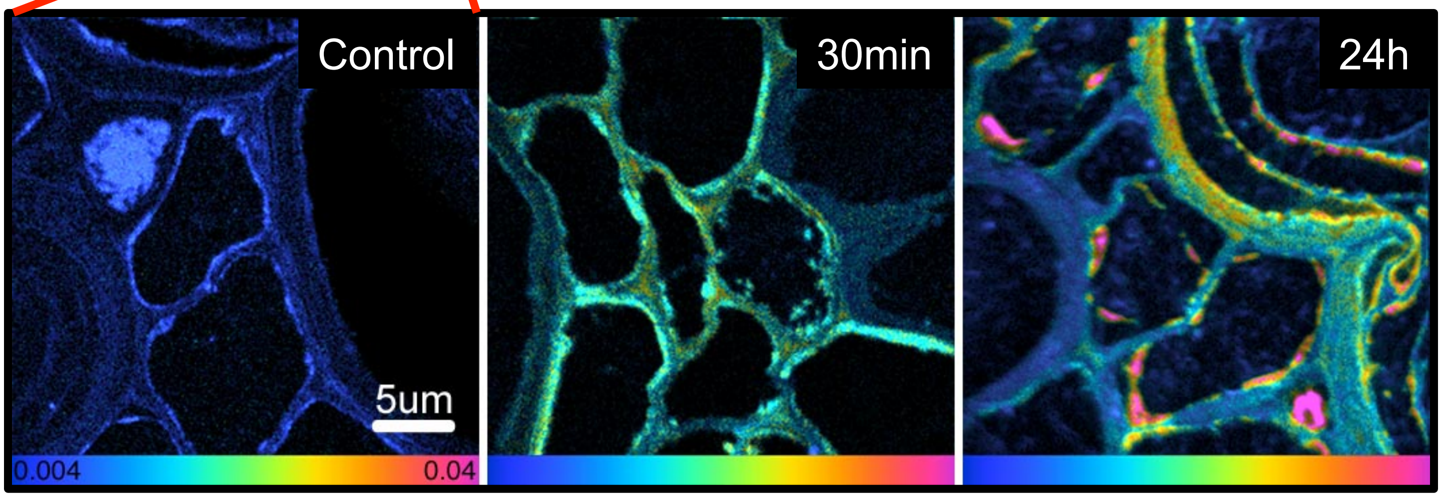
In Situ Mapping of Nutrient Uptake in the Rhizosphere Using Nanoscale Secondary Ion Mass Spectrometry[1[OA]]

Peta L. Clode, Matt R. Kilburn, David L. Jones, Elizabeth A. Stockdale, John B. Cliff III, Anke M. Herrmann[2], and Daniel V. Murphy*



THE UNIVERSITY OF WESTERN AUSTRALIA





$^{15/14}N$ images showing ^{15}N enrichment

Natural

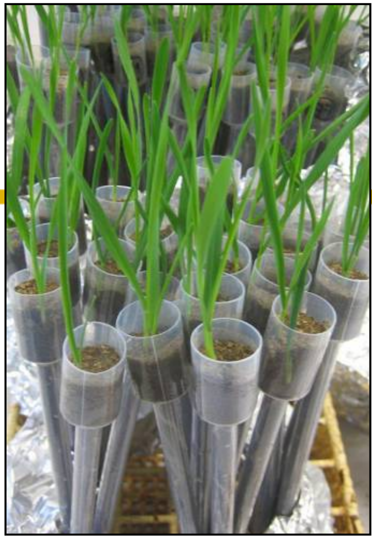
Enriched

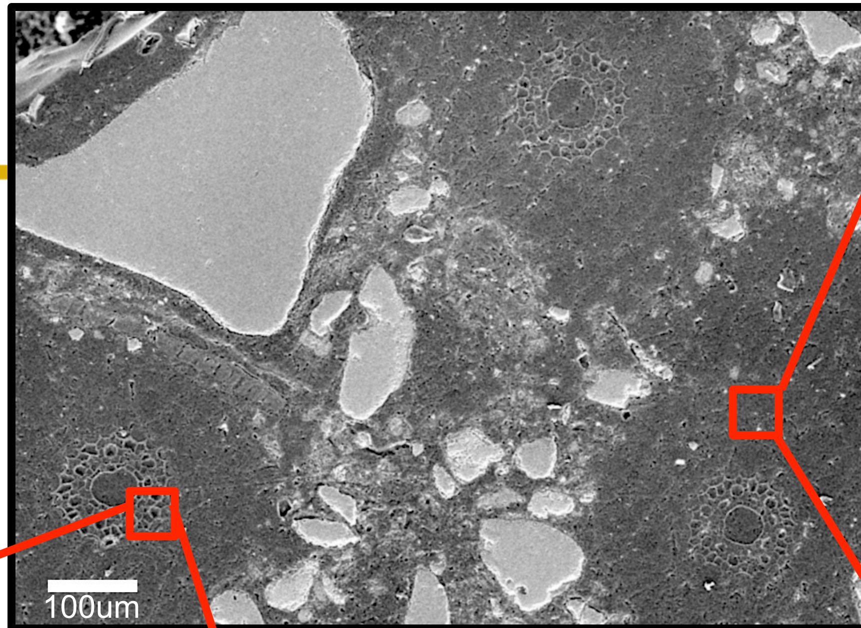
Clode et al. (2009) *Plant Physiology* 151:1751-1757.

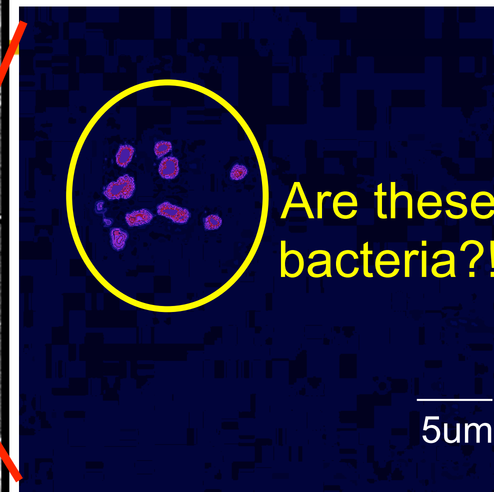
Breakthrough Technologies

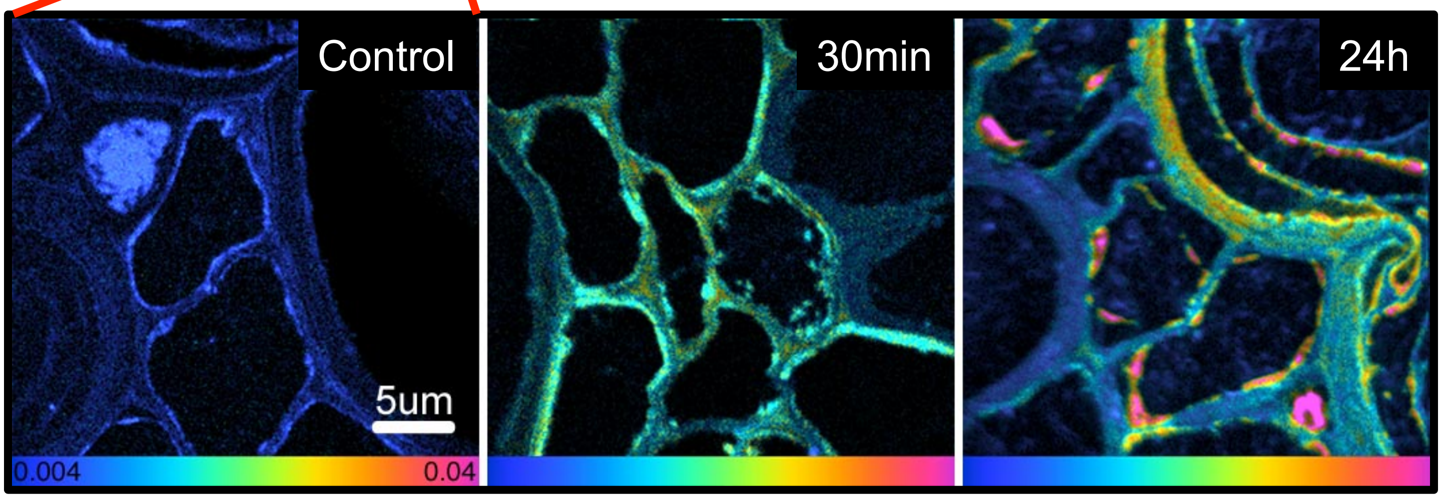
In Situ Mapping of Nutrient Uptake in the Rhizosphere Using Nanoscale Secondary Ion Mass Spectrometry[1[OA]]

Peta L. Clode, Matt R. Kilburn, David L. Jones, Elizabeth A. Stockdale, John B. Cliff III, Anke M. Herrmann[2], and Daniel V. Murphy*









$^{15/14}N$ images showing ^{15}N enrichment

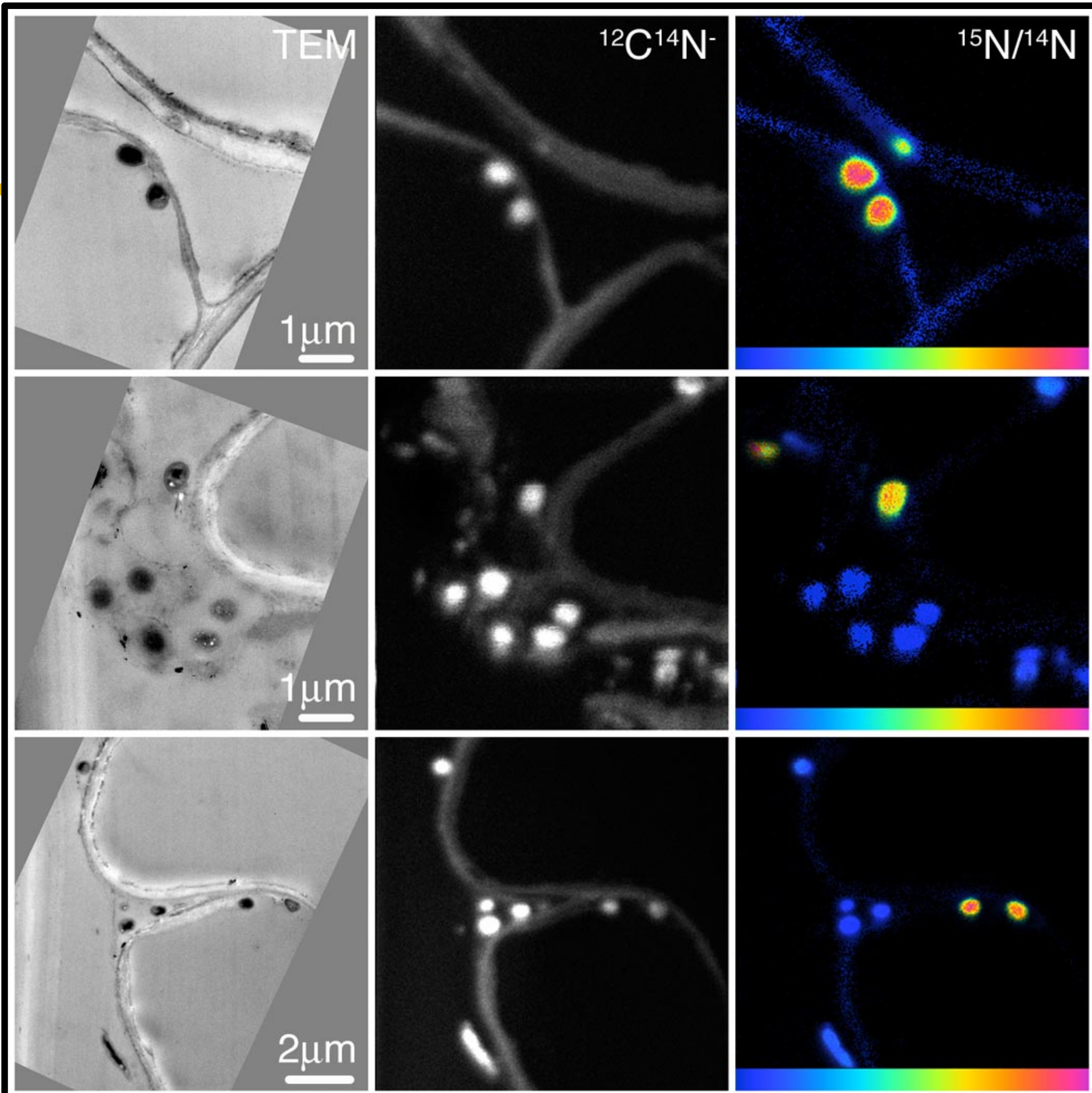
Natural Enriched

Clode et al. (2009) *Plant Physiology* 151:1751-1757.

Breakthrough Technologies

In Situ Mapping of Nutrient Uptake in the Rhizosphere Using Nanoscale Secondary Ion Mass Spectrometry[1][OA]

Peta L. Clode, Matt R. Kilburn, David L. Jones, Elizabeth A. Stockdale, John B. Cliff III, Anke M. Herrmann[2], and Daniel V. Murphy*

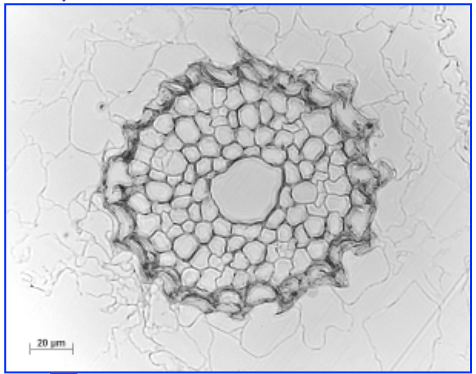


Clode et al. (2009) *Plant Physiology* 151:1751-1757.

THE UNIVERSITY OF WESTERN AUSTRALIA

^{15}N experiment

Remove soil



TEM

NanoSIMS

Stage 3 - 2010

THE UNIVERSITY OF WESTERN AUSTRALIA

Proof of concept

✓ Can we prepare & find ^{15}N-labelled bacteria in a soil core?

Real experiments & real samples

✓ Can we analyse and track ^{15}N competition between bacteria and plant roots in the rhizosphere in soil?

??? What about *^{13}C and/or ^{15}N translocation in mutualistic systems*...?

Unpublished data removed

Looking forward…

THE UNIVERSITY OF WESTERN AUSTRALIA

The main questions:

- Linking activity to species identity directly

  In intact complex systems at single cell level!

- Beyond just isotopes

  What molecule is it…?

- Investigating interfaces

  Correlative / novel methods for looking at mineral-cell interfaces

Looking forward…

The main questions:

- Linking activity to species identity directly

  In intact complex systems at single cell level!

- **Beyond just isotopes**

  What molecule is it…?

- **Investigating interfaces**

  Correlative / novel methods for looking at mineral-cell interfaces

Sample preparation

THE UNIVERSITY OF WESTERN AUSTRALIA

Is always a compromise…

- Sufficient sample quality and structure

  No point analysing at 50 nm resolution if your sample is not preserved adequately at this scale

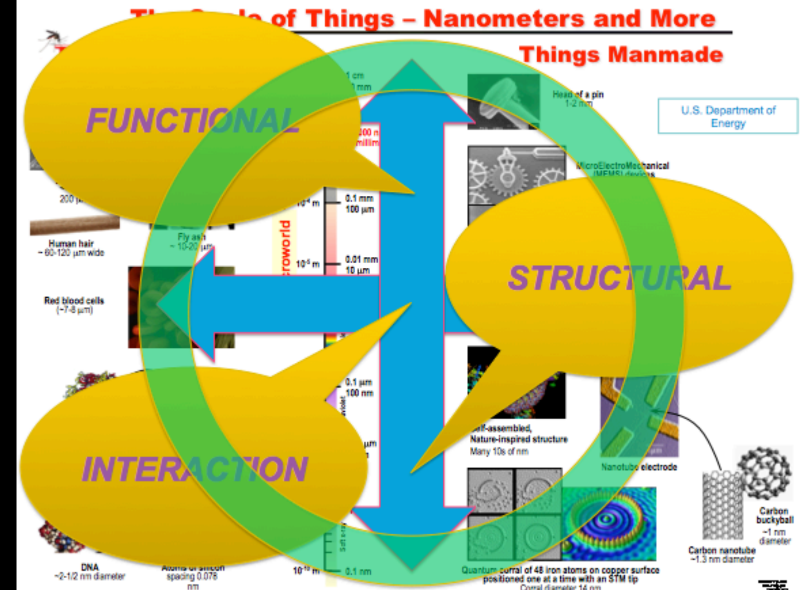
- Preservation of molecules of interest

  What molecule(s)…?

- Tailored to each individual sample and question

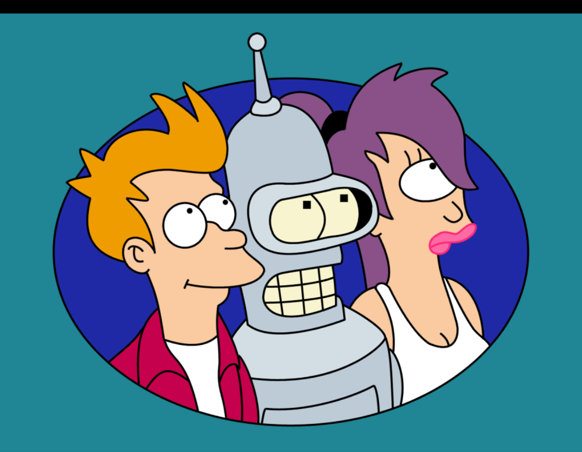
  There is no simple or routine solution

THE FUTURE IS



Very exciting!

…but now, more than ever, complex systems demand inter-disciplinary & correlative approaches…



Acknowledgments

THE UNIVERSITY OF WESTERN AUSTRALIA

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2007; **21**: 29–34
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.2811
RCM

A novel method for the study of the biophysical interface in soils using nano-scale secondary ion mass spectrometry

Anke M. Herrmann[1,5*,†], Peta L. Clode[2], Ian R. Fletcher[2], Naoise Nunan[3], Elizabeth A. Stockdale[4], Anthony G. O'Donnell[5] and Daniel V. Murphy[1]

New Phytologist
Research

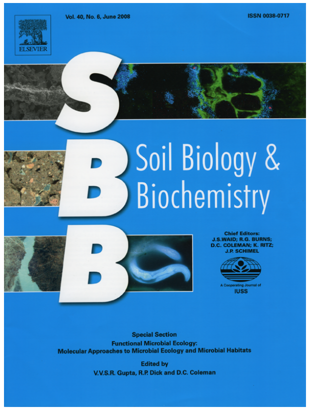
Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation

Christina Kaiser[1,2], Matt R. Kilburn[3], Peta L. Clode[3], Lucia Fuchslueger[2], Marianne Koranda[2], John B. Cliff[3], Zakaria M. Solaiman[1] and Daniel V. Murphy[1]

ELSEVIER
Soil Biology & Biochemistry 39 (2007) 1835–1850
Soil Biology & Biochemistry
www.elsevier.com/locate/soilbio
Review

Nano-scale secondary ion mass spectrometry — A new analytical tool in biogeochemistry and soil ecology: A review article

Anke M. Herrmann[a,f,*], Karl Ritz[b], Naoise Nunan[c], Peta L. Clode[d], Jennifer Pett-Ridge[e], Matt R. Kilburn[d], Daniel V. Murphy[a], Anthony G. O'Donnell[f], Elizabeth A. Stockdale[g]



Research
New Phytologist

Competition between plant and bacterial cells at the microscale regulates the dynamics of nitrogen acquisition in wheat (*Triticum aestivum*)

David L. Jones[1], Peta L. Clode[2], Matt R. Kilburn[2], Elizabeth A. Stockdale[3] and Daniel V. Murphy[4]
[1]Environment Centre Wales, Bangor University, Gwynedd, LL57 2UW, UK; [2]Centre for Microscopy, Characterisation and Analysis, University of Western Australia, Crawley, WA 6009, Australia; [3]School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK; [4]Soil Biology and Molecular Ecology Group, School of Earth and Environment, Institute of Agriculture, University of Western Australia, Crawley, WA 6009, Australia

Plant Physiology

In Situ Mapping of Nutrient Uptake in the Rhizosphere Using Nanoscale Secondary Ion Mass Spectrometry[1[OA]]

Peta L. Clode, Matt R. Kilburn, David L. Jones, Elizabeth A. Stockdale, John B. Cliff III, Anke M. Herrmann[2] and Daniel V. Murphy*

2018 Workshop – Perth?

UWA's PROPOSES TO HOST

The 8th International NanoSIMS Workshop

17 – 19 SEPTEMBER 2018


Thankyou for the invitation and for listening!