RaiseBio
Risk Assessment and Environmental Safety Affected by Compound Bioavailability in Multiphase Environments

Marie Curie Actions
Human Resources and Mobility Activity

Biotransformation of ibuprofen in soil: a new insight into non-extractable residue formation

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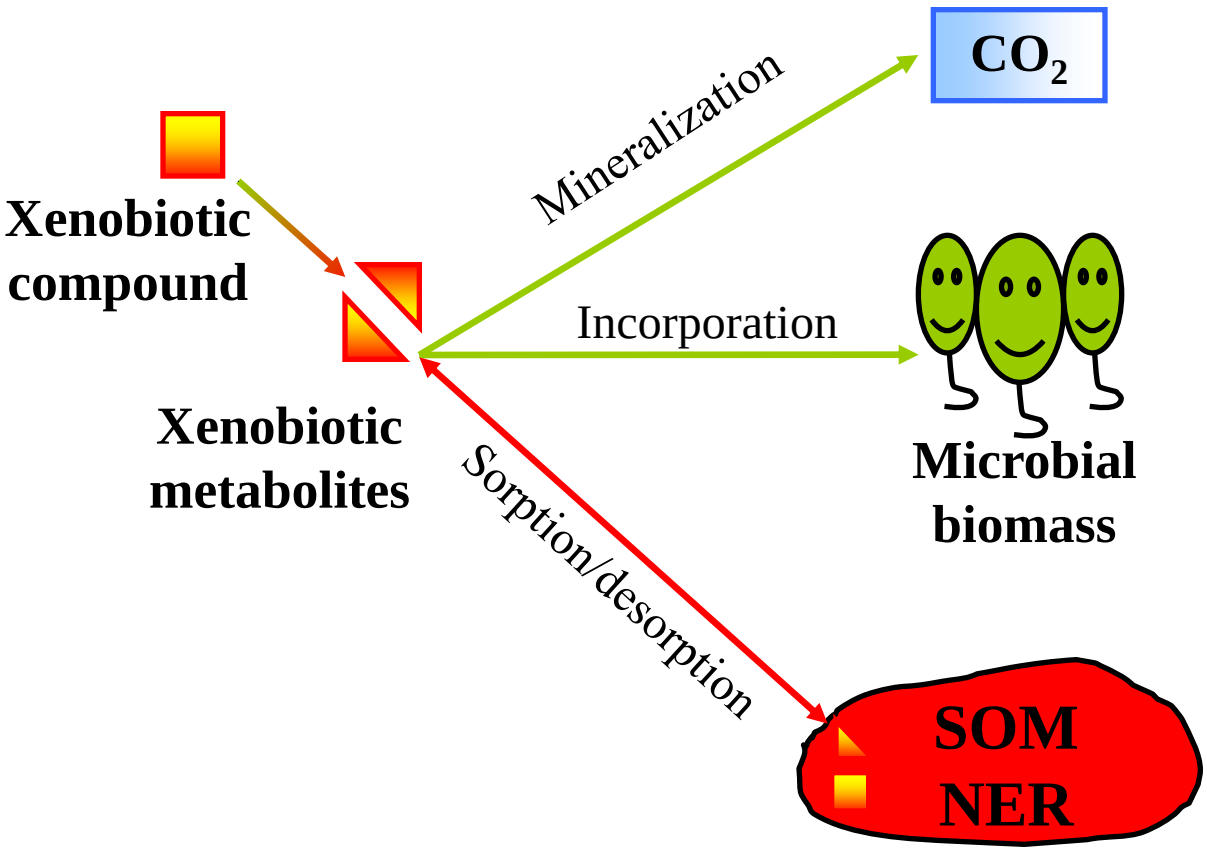
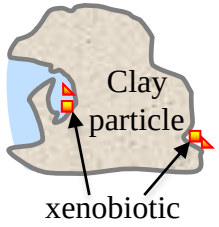
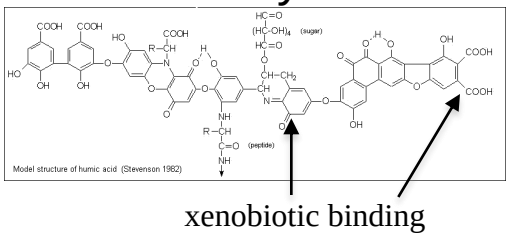
MASS BALANCE OF A XENOBIOTIC IN SOIL



1. Sequestered NER



2. Chemically bound



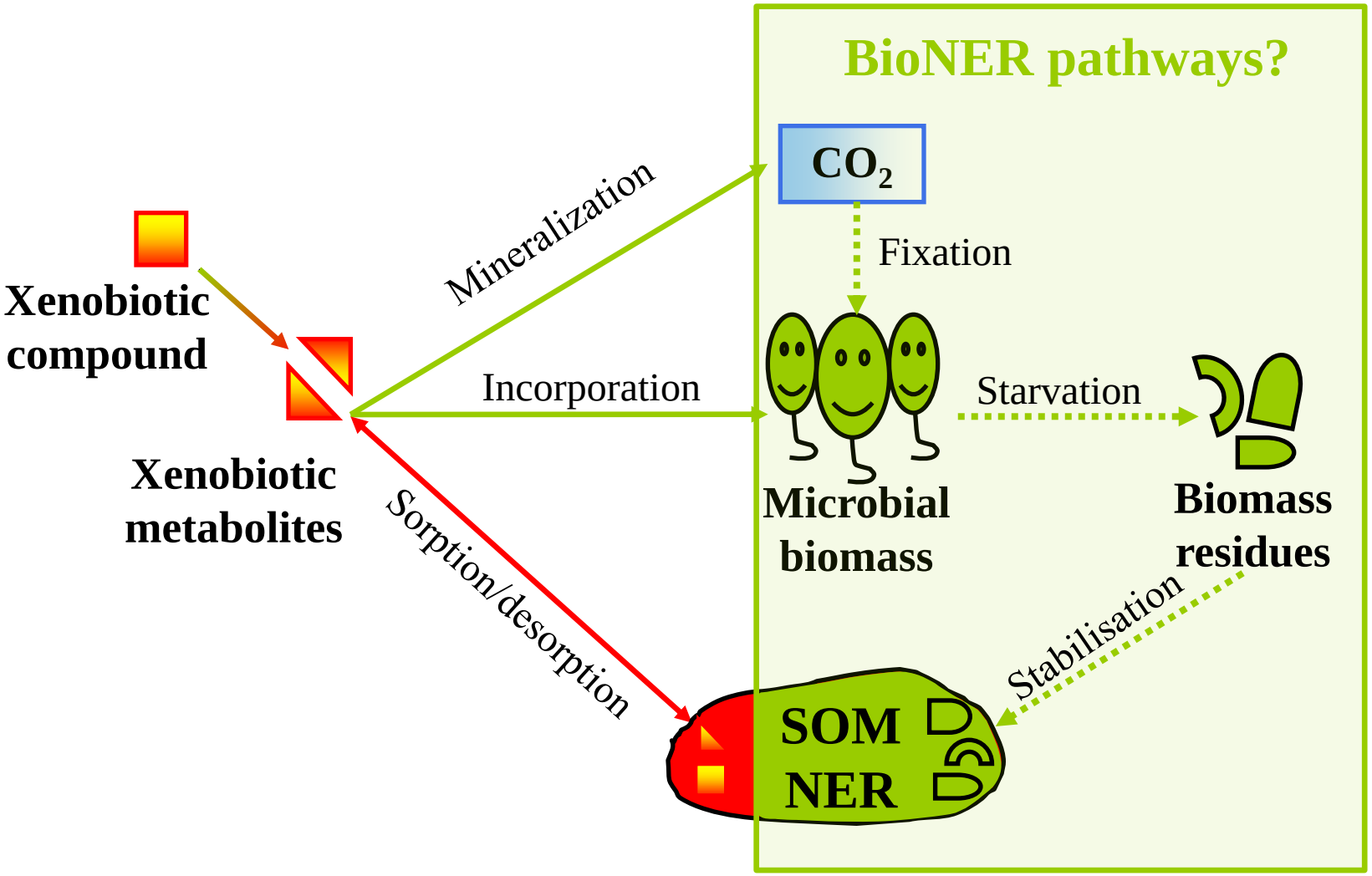
Simple systems!

Soils: mostly quantification!

NER structure??

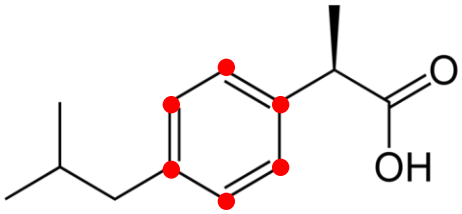
Risk??

BioNER FROM A XENOBIOTIC IN SOIL



IBUPROFEN (IBU)

- Anti-inflammatory and analgesic drug
- Most commonly consumed drug
- Detected in effluents and sewage sludge
- Biodegraded in soil
- High NER content → NER structure?? Risk??



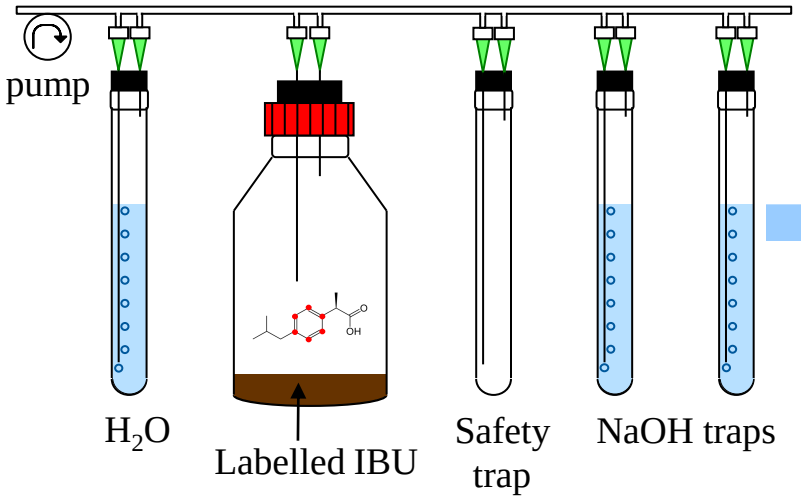
$^{13}C_6$-Ibuprofen

M_w - 206.28 g/mol

H_2O solubility - 21 mg/L

log K_{ow} - 3.5

$^{13}C_6$-IBU EXPERIMENT



CO_2
(TIC;
GC-C-IRMS)

- Darkness, 20ºC; 60% of WHC
- $^{13}C_6$-IBU: 20 mg/kg
- 2, 4, 14, 28, 59 and 90 days
- 21% clay, 68% silt, 11% sand
- Abiotic, ^{13}C-abundance controls

$^{13}C_6$-IBU RESIDUES

Non-extractable residues (NER)
(EA-C-IRMS)

Extractable compound residues
(parent compound + primary metabolites)

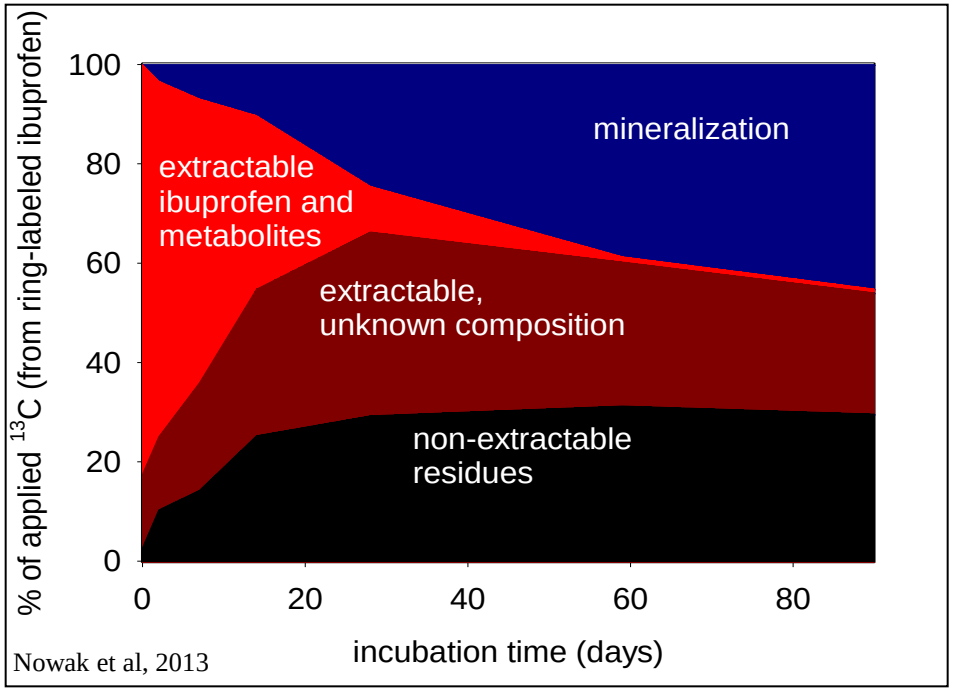
GC/MS;
GC-C-IRMS

BioNER analyses

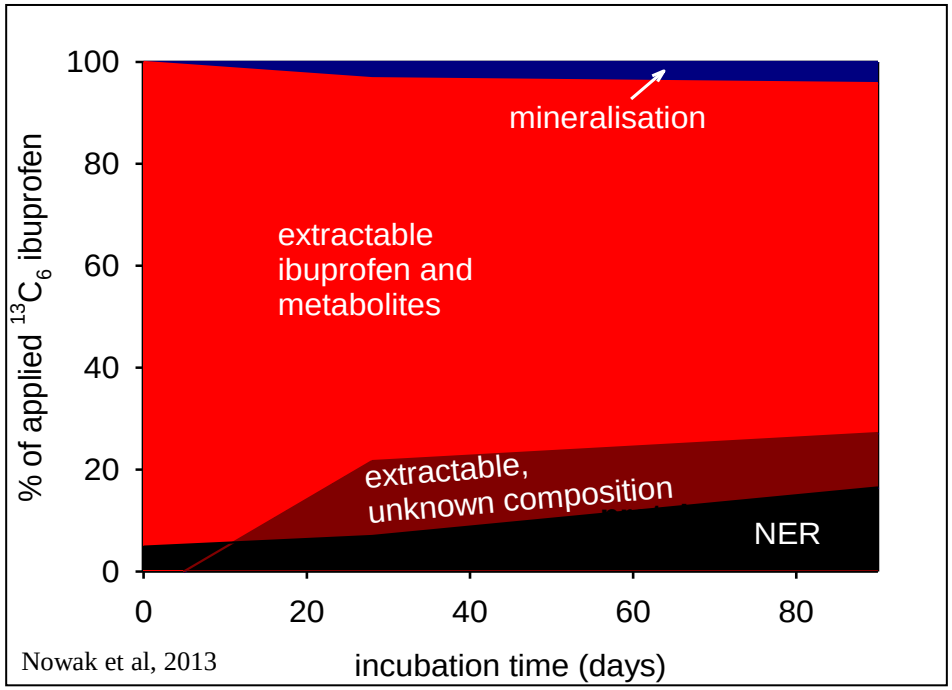
- **Living biomass:**
  (**PLFA** and **bioAA**)
- **Total in soil:**
  (non-living + living biomass:
  tFA and **tAA**)

^{13}C-MASS BALANCE ($^{13}C_6$-IBU)

BIOTIC



ABIOTIC

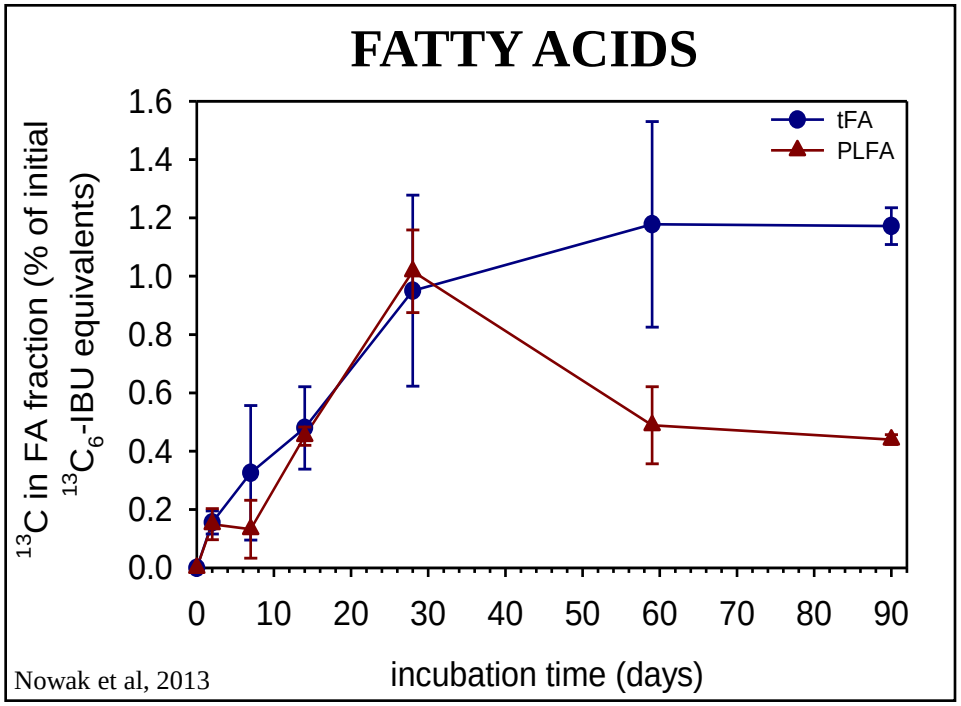


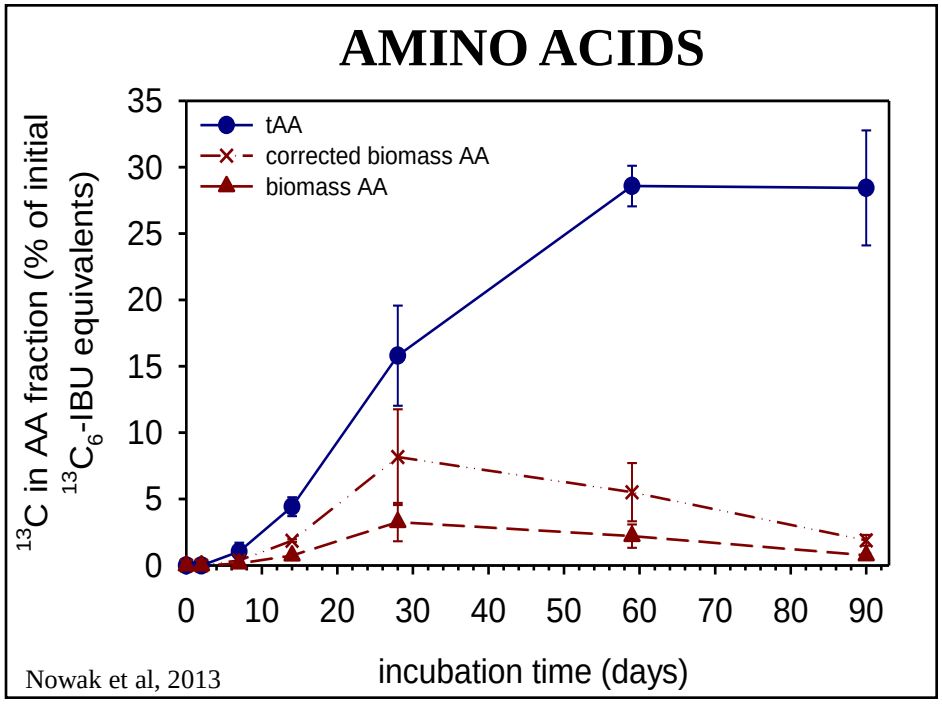
- Mineralisation: high
- NER: high → **bioNER**?
- ^{13}C-IBU + metabolites:↓

- Mineralisation: low
- NER: low → time dependant
- ^{13}C-IBU + metabolites: high

NER = microbial activity!

INCORPORATION OF ^{13}C INTO FA and AA

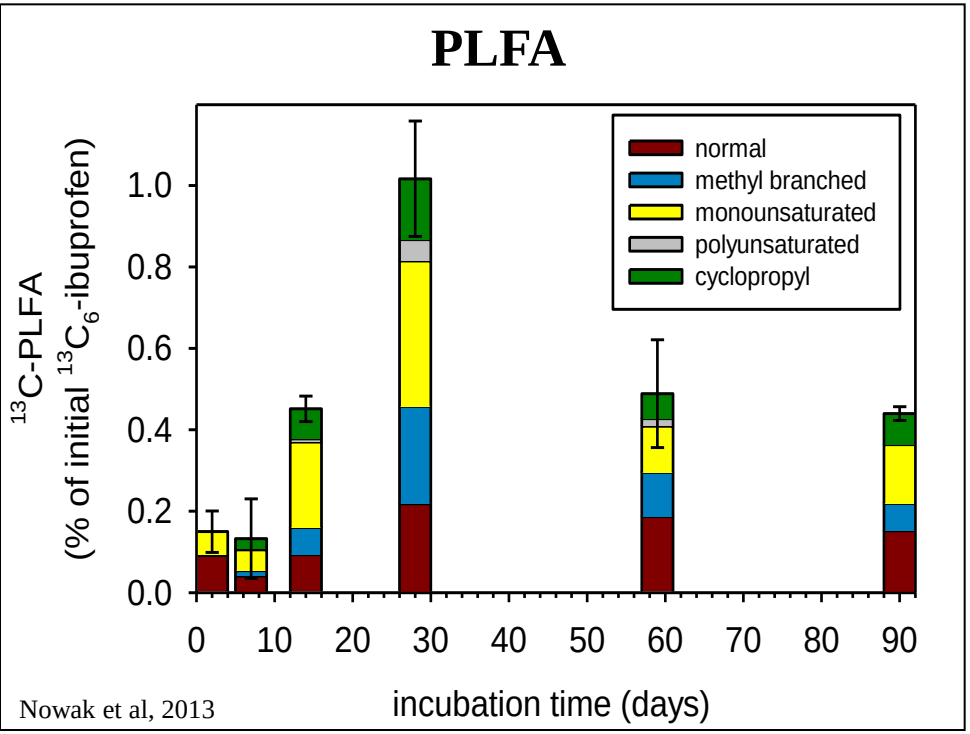


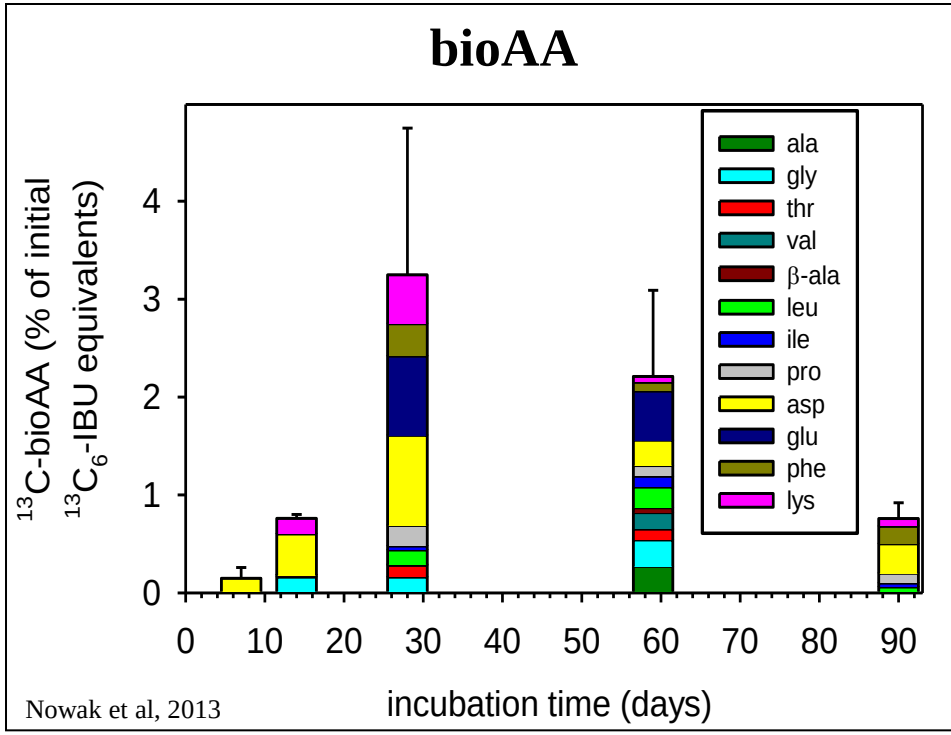


- PLFA: fast
- PLFA:↓

- bioAA: fast
- bioAA:↓
- **tAA: 27%!**

^{13}C INCORPORATION INTO BIOMASS

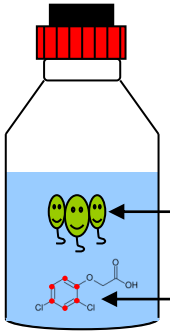




- G^- markers: initial degraders
- G^+ markers: later phase
- Starvation marker:↑over time

- Aspartate: initially → CO_2 fixation
- diverse bioAA: later phase

CALCULATION OF TOTAL BioNER



Cupriavidus necator JMP 134

$^{13}C_6$-2,4-D

- ^{13}C in **PLFA** and **bioAA**
- Total ^{13}C in biomass

GC/MS;
EA-C-IRMS
GC-C-IRMS

Incorporation of ^{13}C into the biomass of *C. necator* grown on $^{13}C_6$-2,4-D

Name	Incubation time (days) [% of $^{13}C_6$-2,4-D]			
	2	3	7	14
Biomass	9.2 (±1.5)	10.0 (±1.5)	14.5 (±1.8)	17.4 (±0.06)
PLFA	0.5 (±0.02)	0.6 (±0.01)	0.8 (±0.05)	0.6 (±0.04)
bioAA	4.7 (±0.2)	6.1 (±0.3)	7.3 (±0.2)	8.1 (±0.4)
Biomass/PLFA	18.4	17	18	29
Biomass/AA	1.9	1.6	2	2.1

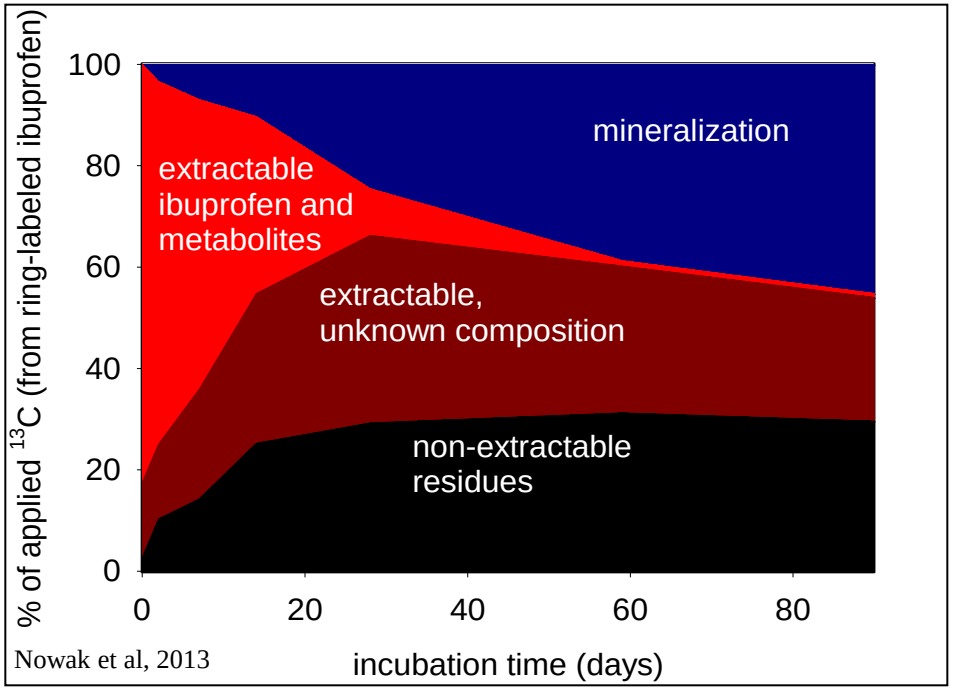
Conversion factor of ~ 2 for tAA (proteins)

tFA instable (Nowak et al, 2011)! THUS tAA → calculation

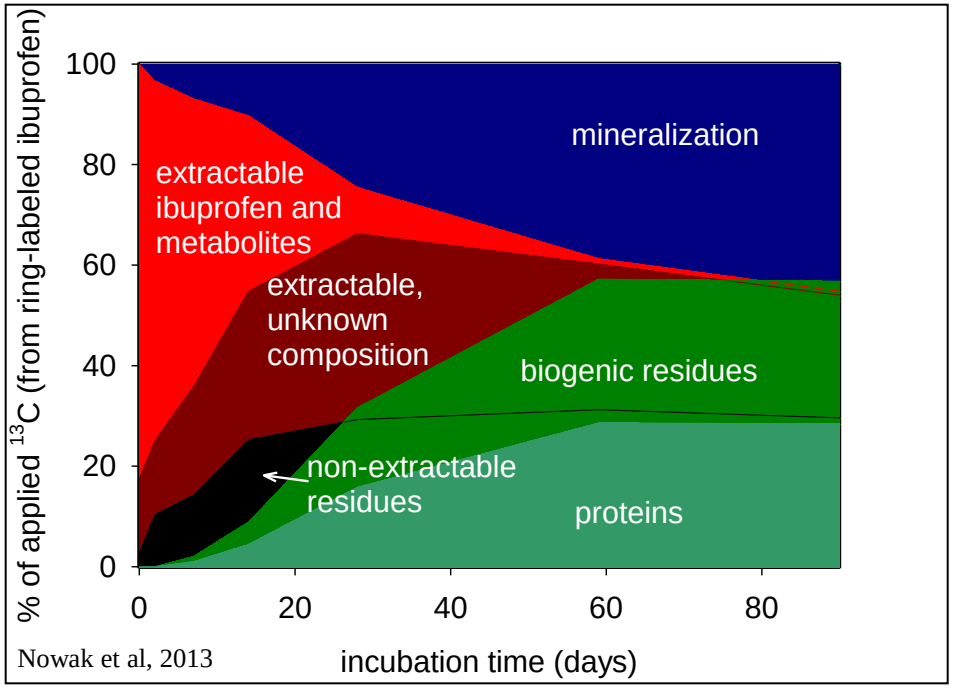
Nowak et al, 2011

^{13}C-MASS BALANCE ($^{13}C_6$-IBU)

General mass balance



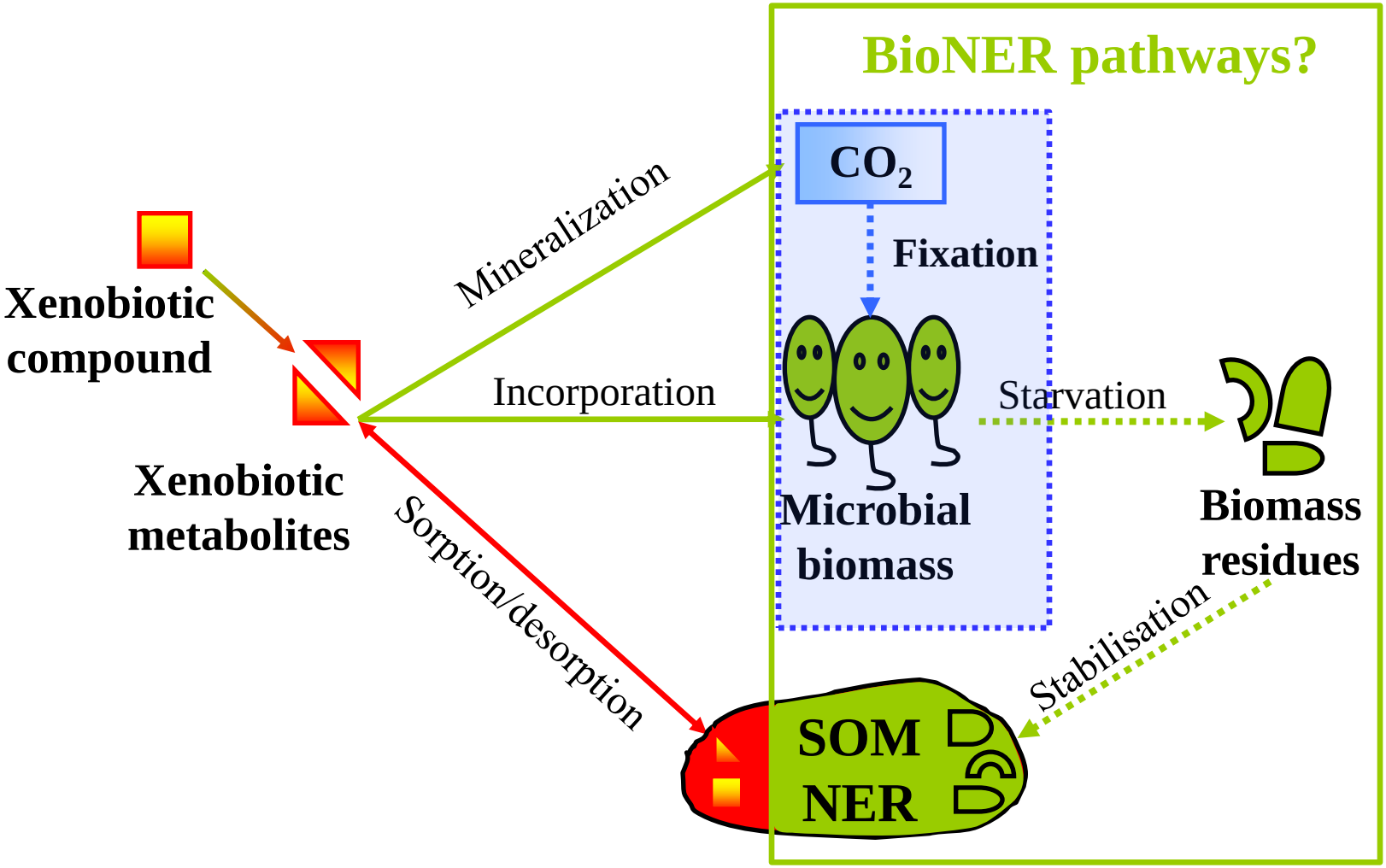
New mass balance incl. BioNER



- extractable (unknown composition): bioNER?

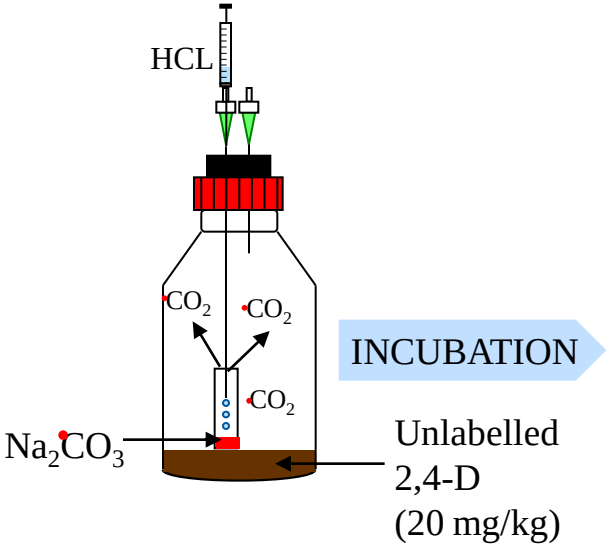
Nearly all NER biogenic!

BioNER FROM CO_2 FIXATION

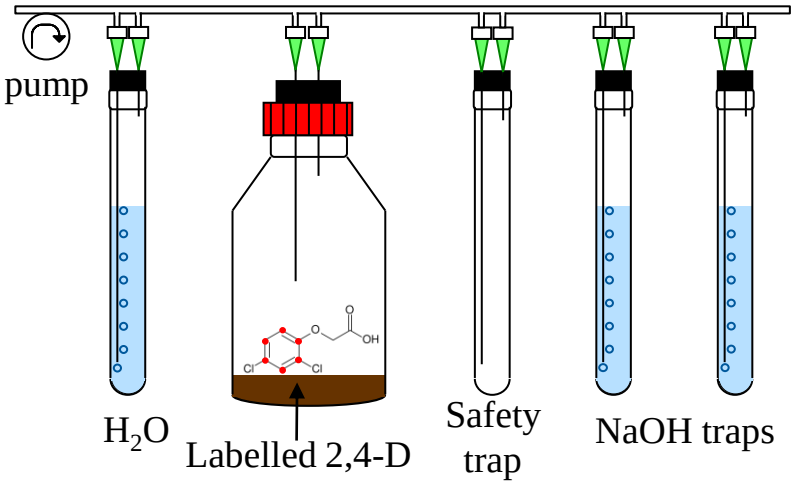


CO_2 FIXATION EXPERIMENT

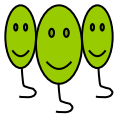
$^{13}CO_2$ experiment



$^{13}C_6$-2,4-D experiment



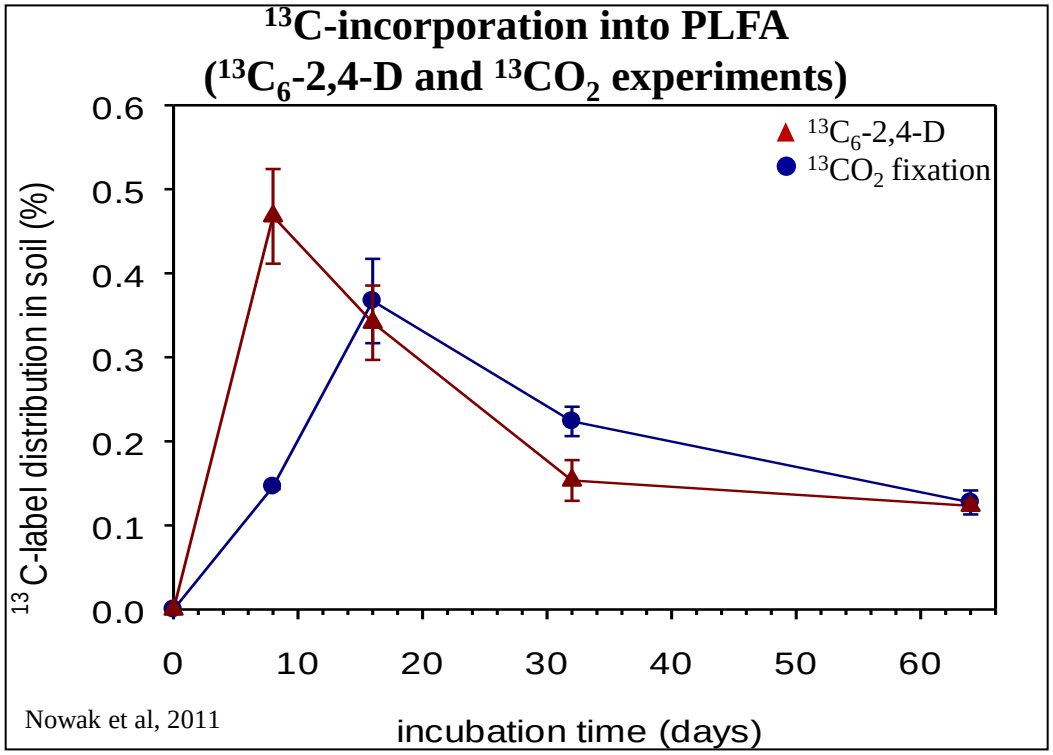
^{13}C-LABEL ANALYSES

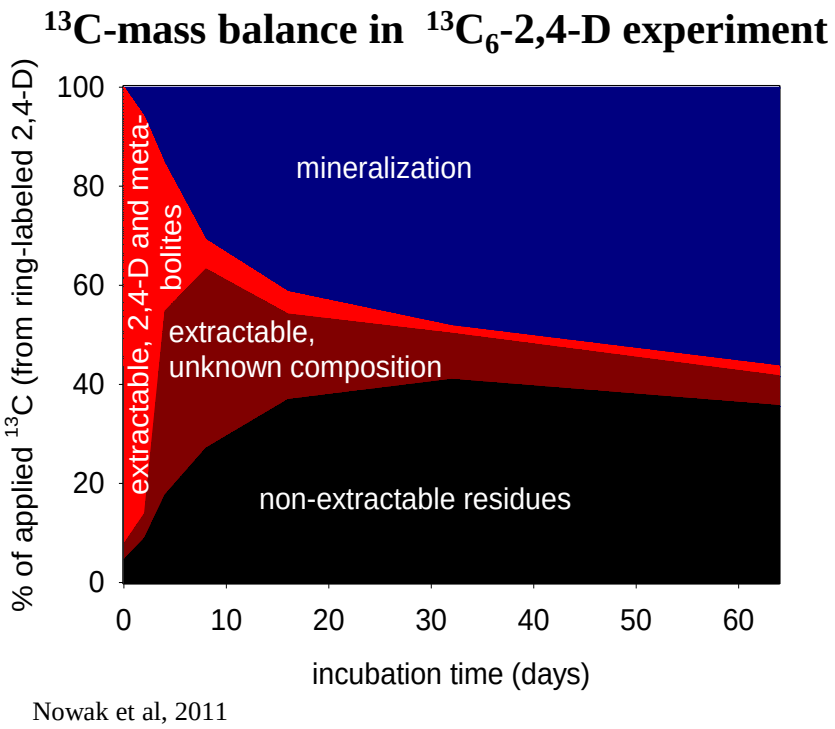
BIONER analyses 

- **Living biomass:** (**PLFA**)

GC/MS;
GC-C-IRMS

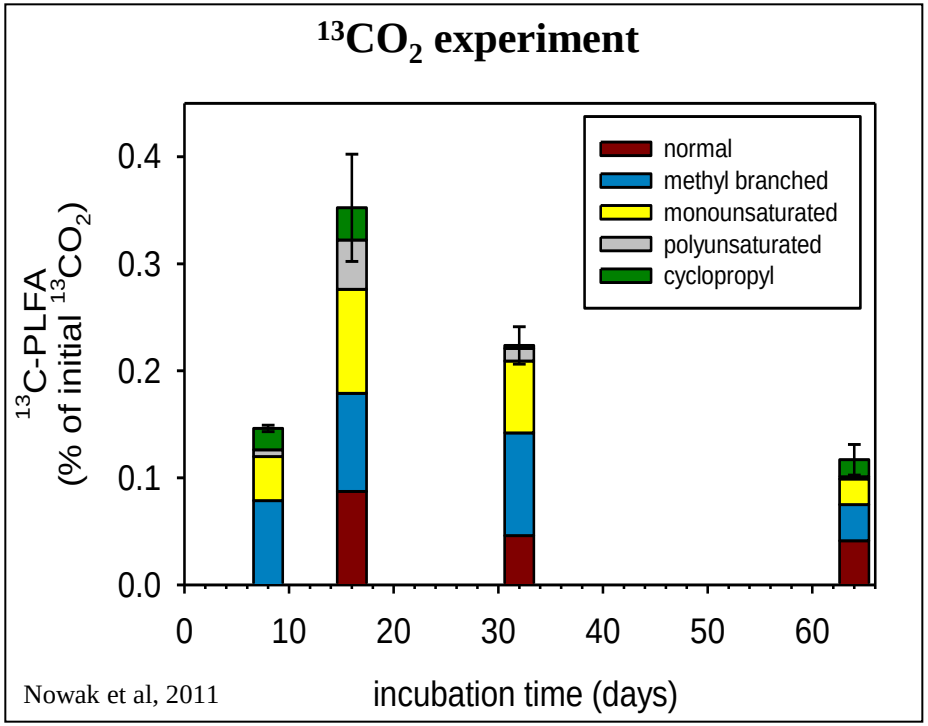
INCORPORATION OF ^{13}C INTO PLFA

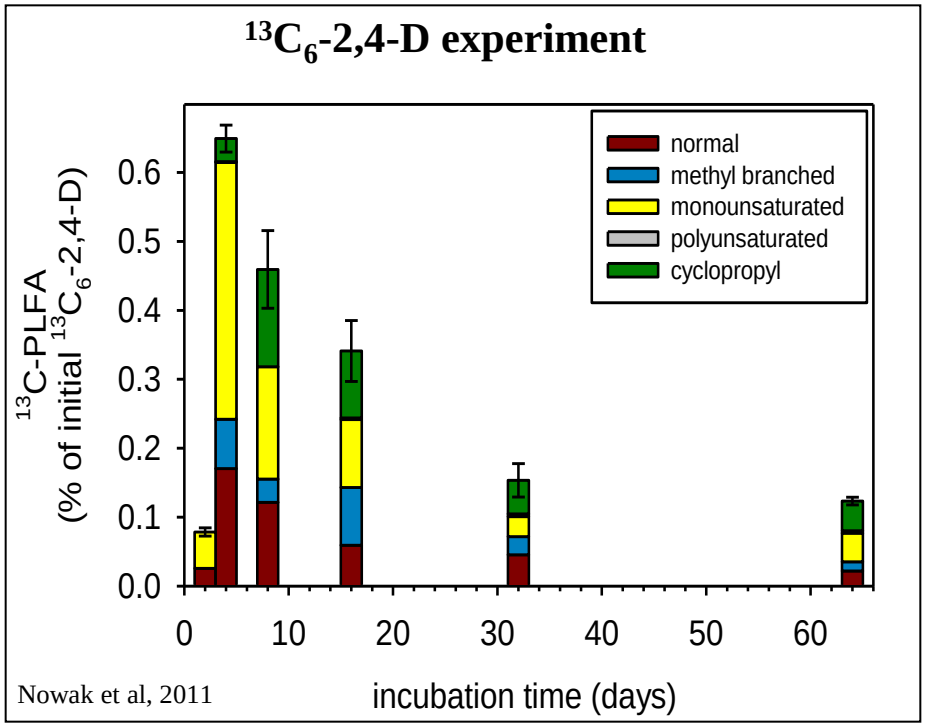




- $^{13}CO_2$ fixation (day 16)
- PLFA: decline

^{13}C IN PLFA CLASSES





- G^+ markers: initially

- G^- markers: initially

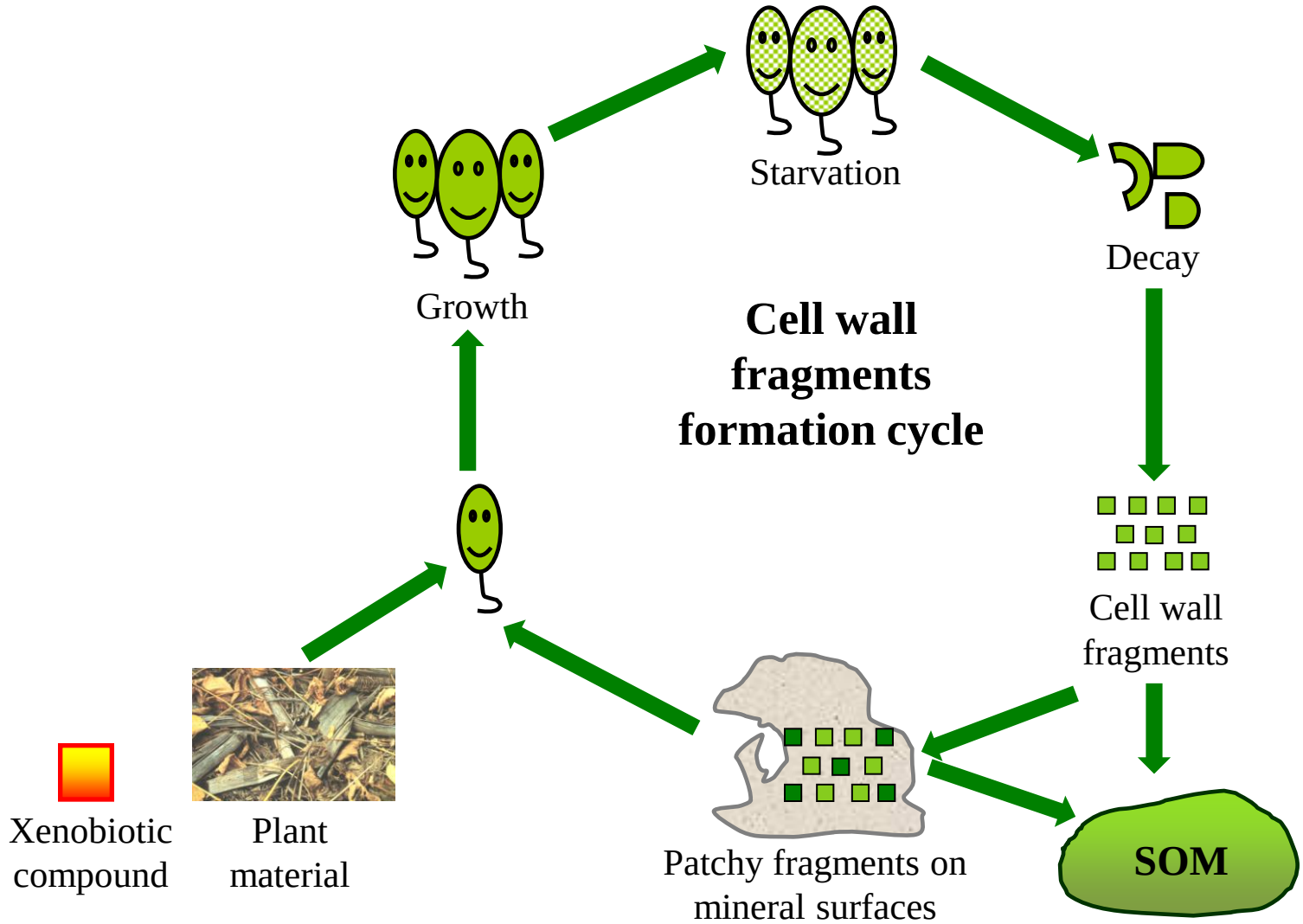
FINAL REMARKS

- NER from $^{13}C_6$-IBU biogenic = **no risk!**
- NER in abiotic soil low
- tAA high
- bioNER from xenobiotic and CO_2

HOWEVER:

- no biodegradation → xenobiotic NER
- bioNER → biodegradation (↑CO_2) → SOM formation

SOM FORMATION



Miltner et al, 2012

FINAL REMARKS

- NER from $^{13}C_6$-IBU biogenic = **no risk!**
- NER in abiotic soil low
- tAA high
- bioNER from xenobiotic and CO_2

HOWEVER:

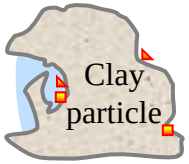
- no biodegradation → xenobiotic NER
- bioNER → biodegradation (↑CO_2) → SOM formation

AND:

- **Biotic vs abiotic NER formations (3 types of NER)!**

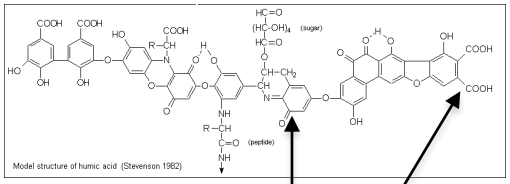
NER CLASSIFICATION

- **type I: sequestered NER**:



- reversible
- remobilisation → risk for the environment

- **type II: chemically bound (covalent bonding)**



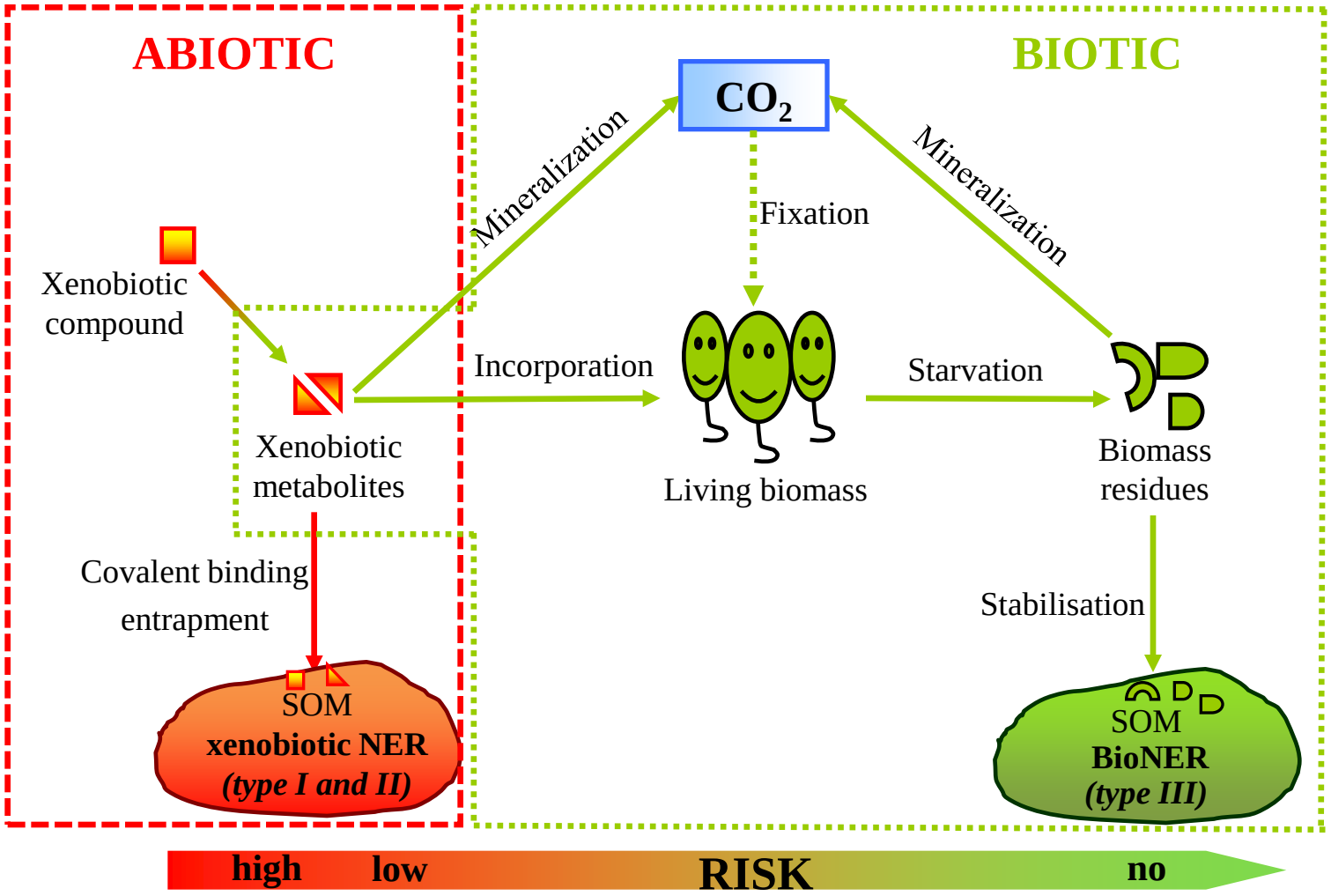
xenobiotic binding

- irreversible
- low risk for environment

- **type III: bioNER**

- biomolecules (amino acids, fatty acids) → SOM
- no risk

ABIOTIC vs BIOTIC NER FORMATION



Kästner et al, in press

FURTHER RESEARCH

- BioNER from other contaminants
  (different structure, slower degradation)
- AA: 50%, FA: 5% of BioNER: other components?
- New risk assessment including bioNER formation

THANK YOU FOR YOUR ATTENTION!

EXTRACTION OF BIONER

FATTY ACIDS

- **Living biomass:** (**PLFA**)

Extraction:
PB/MeOH/CH_3Cl
(0.8/2/1, v:v)

- **Phospholipids (PLFA)**
- Neutral lipids
- Glycolipids

Purification: silica gel ⇒ **PLFA**
(CH_3Cl, ACN, MeOH)

Derivatization:
MeOH/TMCS; 9:1, v:v

- **Total in soil:** (non-living + living biomass: **tFA** and **tAA**)

Derivatization:
(MeOH/TMCS; 9:1, v:v)

Purification: silica gel
(diethyl ether)

AMINO ACIDS

- **Living biomass:** (**bioAA**)

Biomass extraction:
chelating cation exchange resin+
sodium deoxycholate/Polyethylenglycol 600
(0.1%/2.5%)

- **Total in soil:** (non-living + living biomass: **tFA** and **tAA**)

Hydrolysis: 6M HCl, 110ºC

Purification: DOWEX 50W-X8
(oxalic acid, 0.01M HCl, H_2O, 2.5M NH_4OH)

Derivatization:
(Isopropanol/acetylchloride;
DCM/Trifluoroacetic acid anhydride)

Purification:
(PB:CH_3Cl)

GC/MS;
GC-C-IRMS