

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

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MASS BALANCE OF A XENOBIOTIC IN SOIL

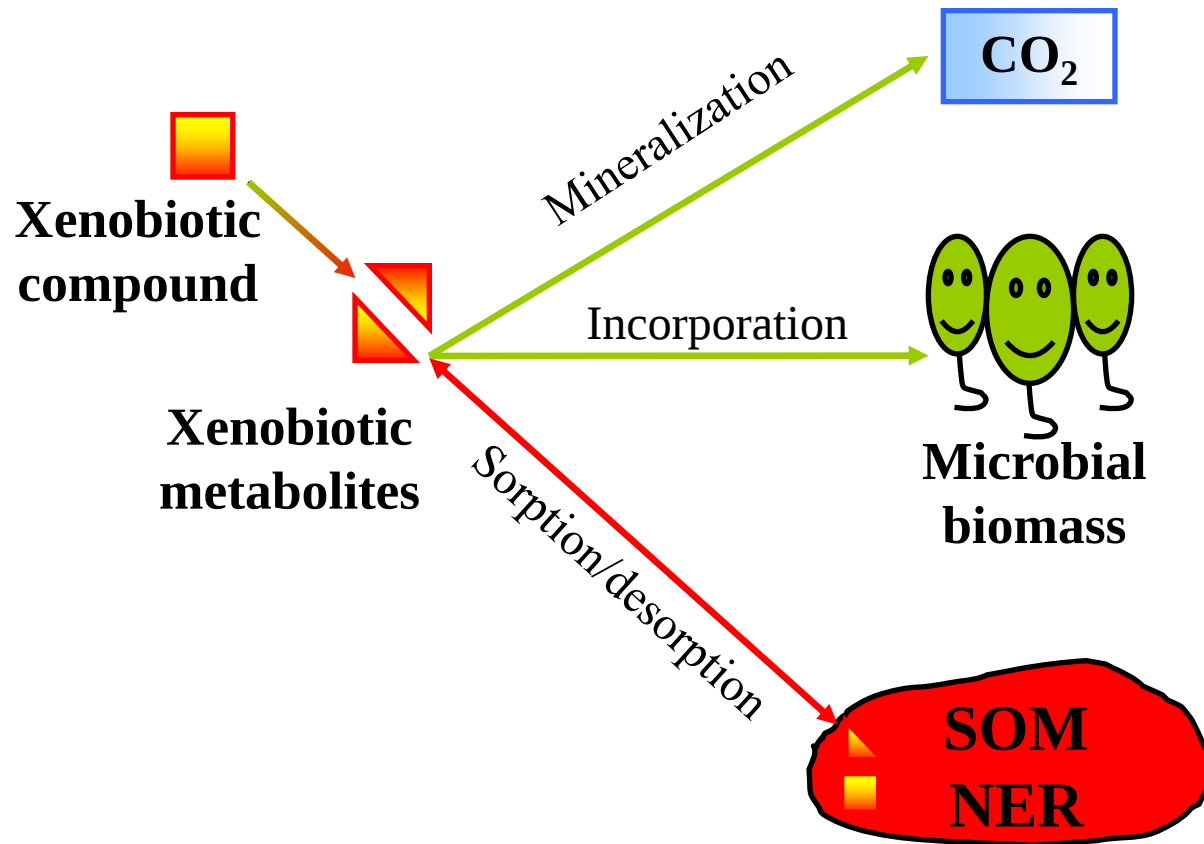
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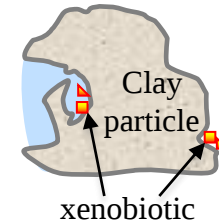
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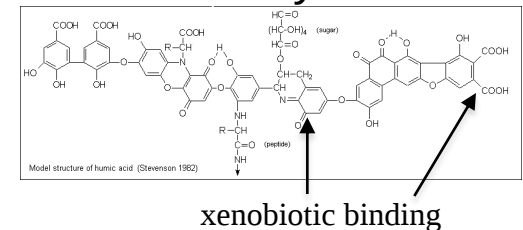
PLANS...



1. Sequestered NER



2. Chemically bound



Simple systems!

**Soils: mostly
quantification!**

NER structure??

Risk??

BioNER FROM A XENOBIOTIC IN SOIL

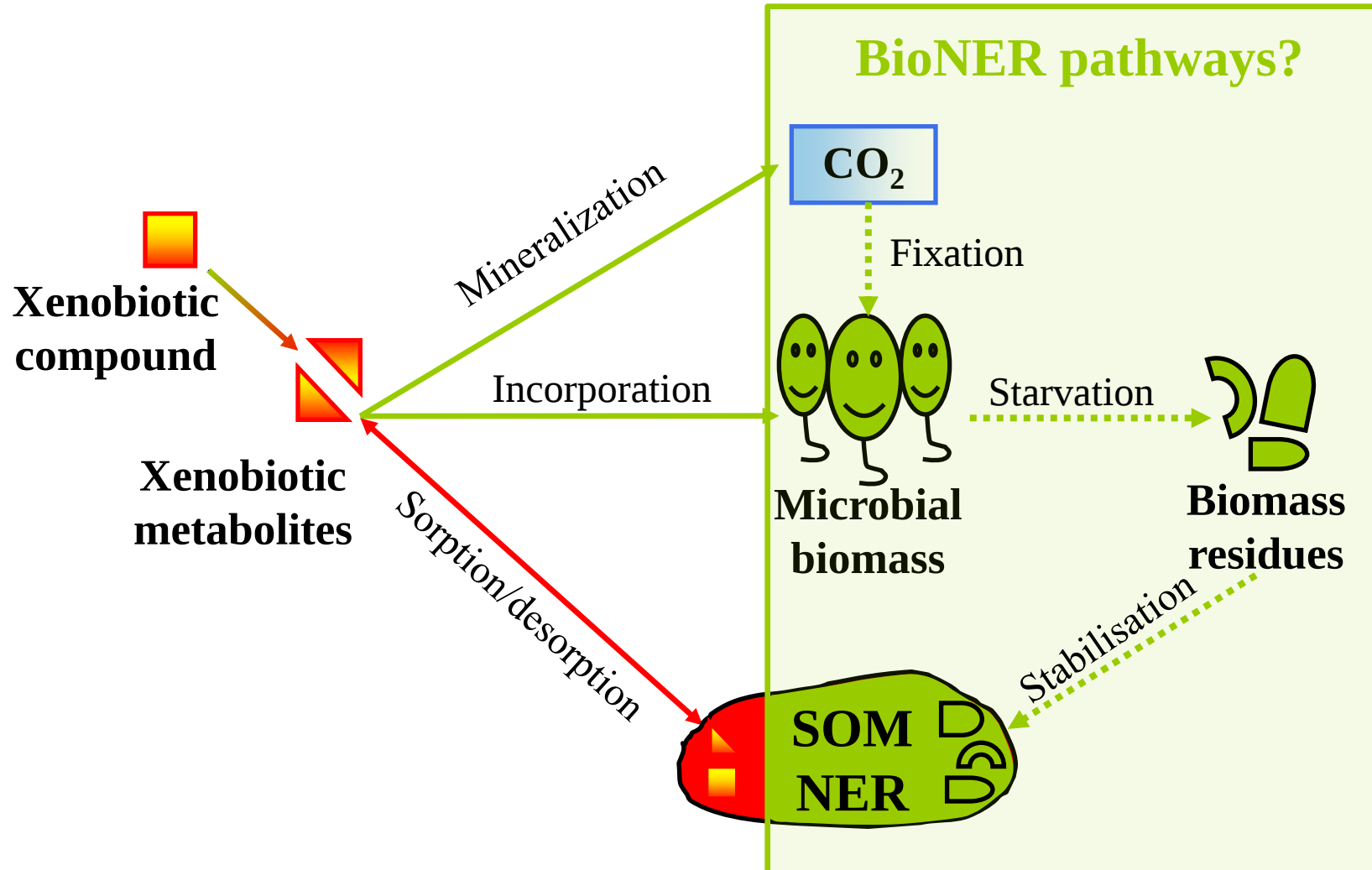
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IBUPROFEN (IBU)

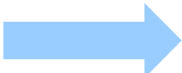
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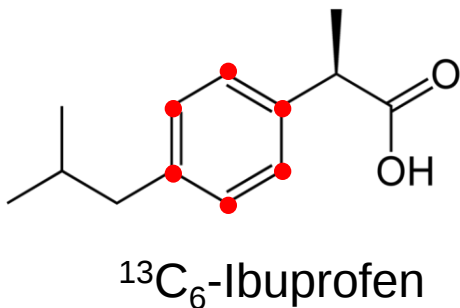
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- Anti-inflammatory and analgesic drug
- Most commonly consumed drug
- Detected in effluents and sewage sludge
- Biodegraded in soil
- High NER content  NER structure?? Risk??



M_w - 206.28 g/mol

H_2O solubility - 21 mg/L

$\log K_{ow}$ - 3.5

$^{13}\text{C}_6$ -IBU EXPERIMENT

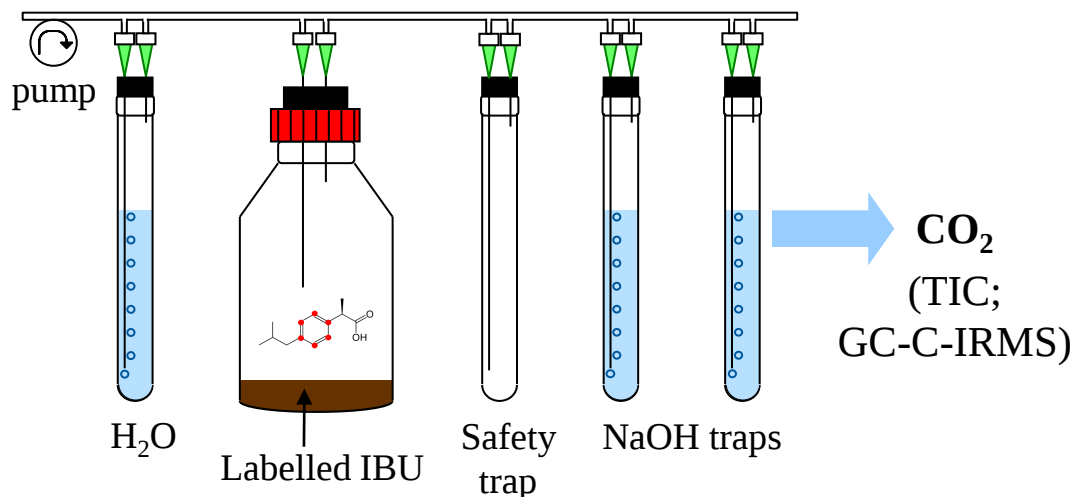
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- Darkness, 20°C; 60% of WHC
- $^{13}\text{C}_6$ -IBU: 20 mg/kg
- 2, 4, 14, 28, 59 and 90 days
- 21% clay, 68% silt, 11% sand
- Abiotic, ^{13}C -abundance controls

$^{13}\text{C}_6$ -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 ($^{13}\text{C}_6$ -IBU)

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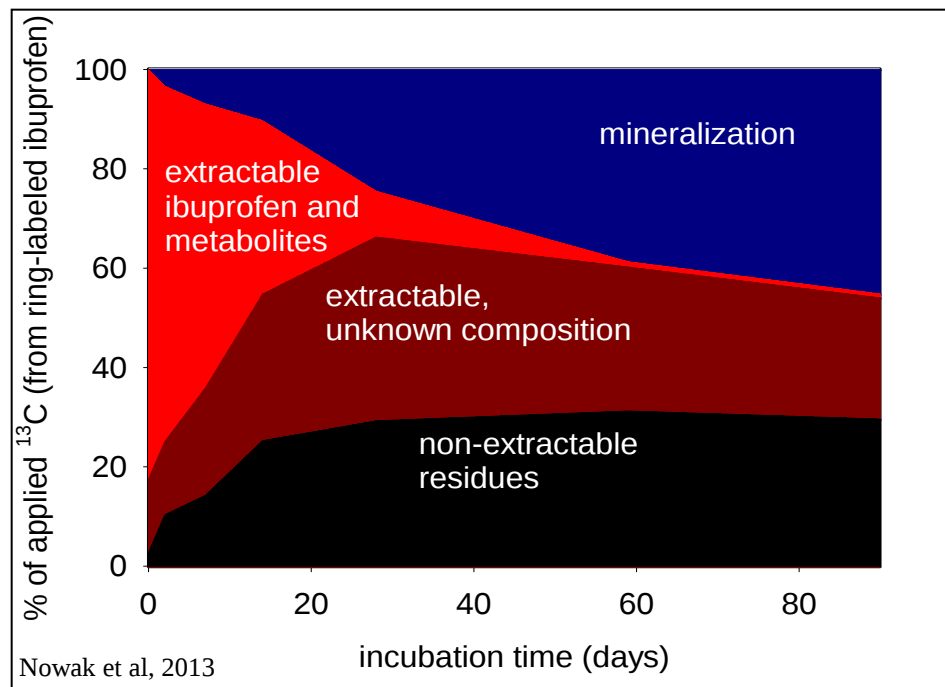
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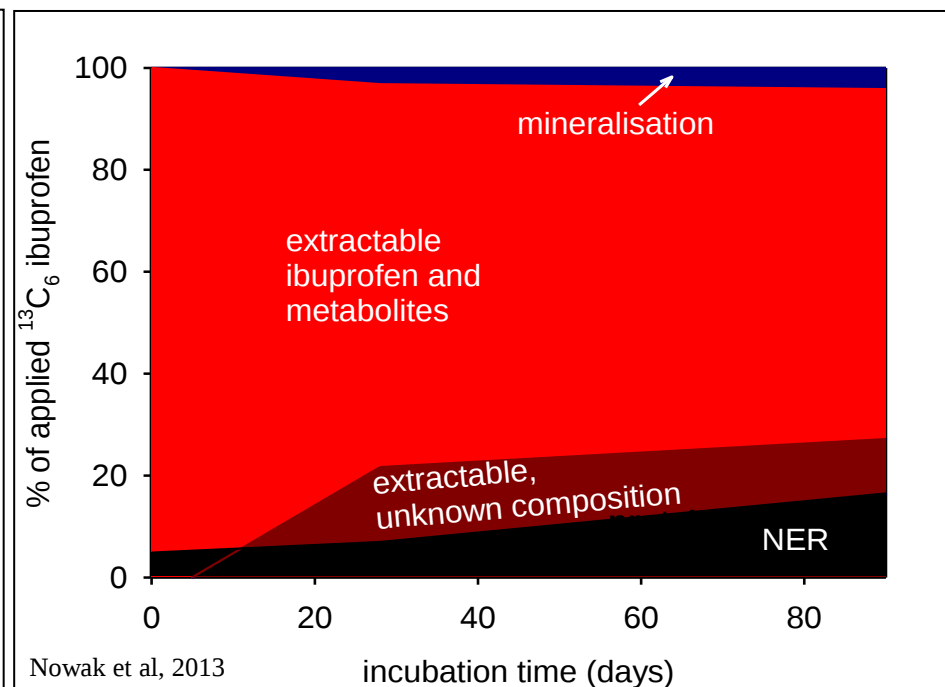
CONCLUSIONS

PLANS...

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

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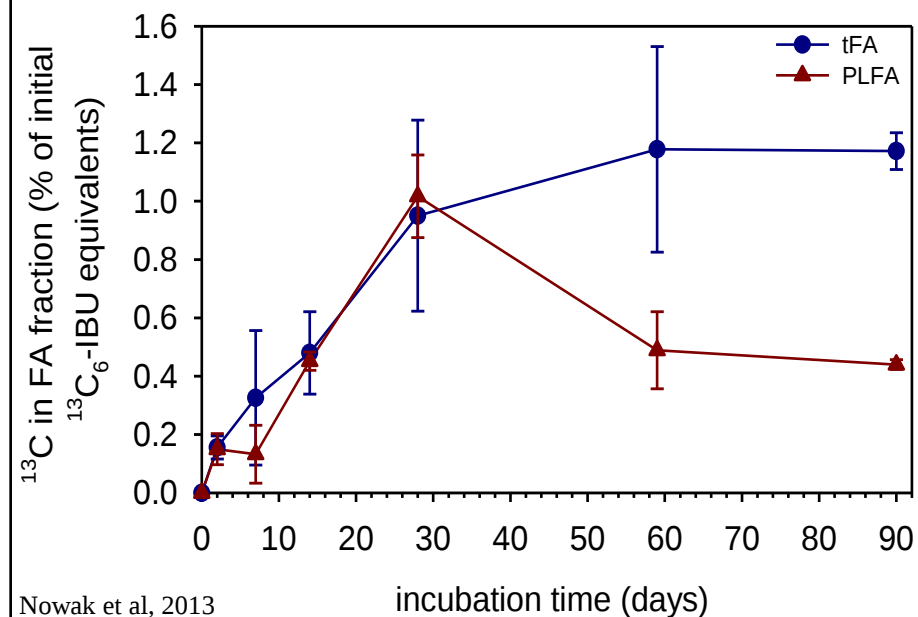
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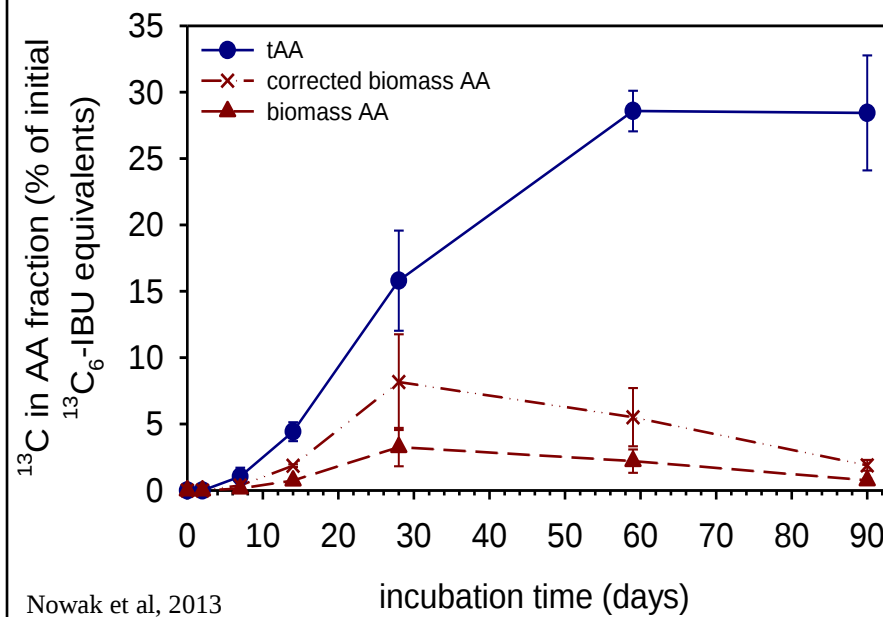
FATTY ACIDS



Nowak et al, 2013

- PLFA: fast
- PLFA:↓

AMINO ACIDS



Nowak et al, 2013

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

^{13}C INCORPORATION INTO BIOMASS

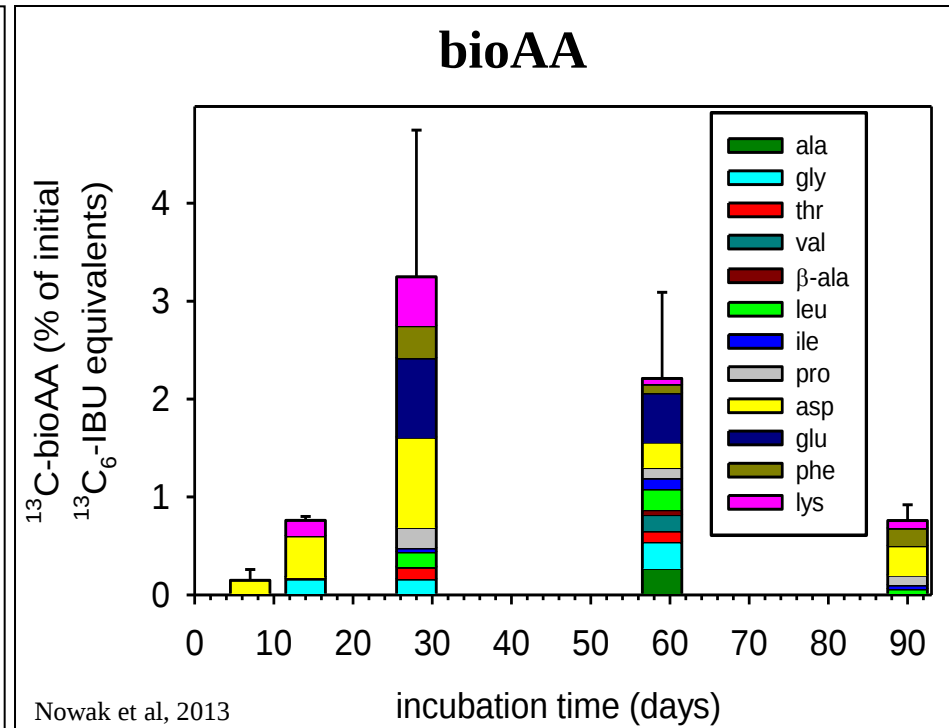
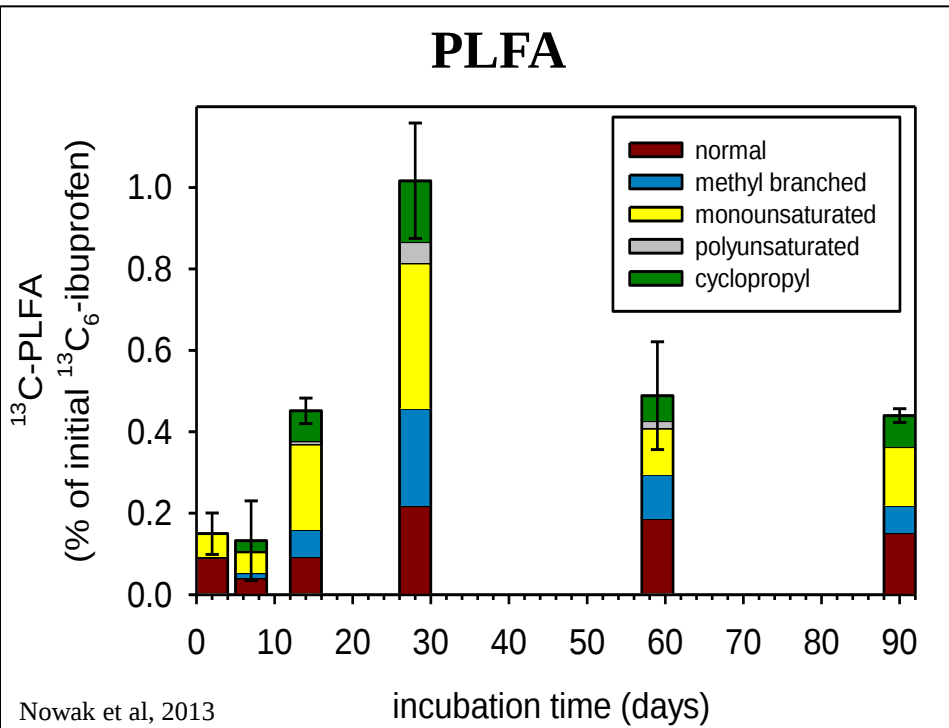
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- G^- markers: initial degraders
- G^+ markers: later phase
- Starvation marker: $\uparrow$ over time

- Aspartate: initially $\rightarrow \text{CO}_2$ fixation
- diverse bioAA: later phase

CALCULATION OF TOTAL BioNER

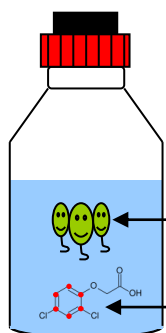
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*Cupriavidus
necator* JMP 134

$^{13}\text{C}_6\text{-2,4-D}$

- ^{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 $^{13}\text{C}_6\text{-2,4-D}$

Name	Incubation time (days) [% of $^{13}\text{C}_6\text{-2,4-D}$]			
	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 $\rightarrow$ calculation

^{13}C -MASS BALANCE ($^{13}\text{C}_6$ -IBU)

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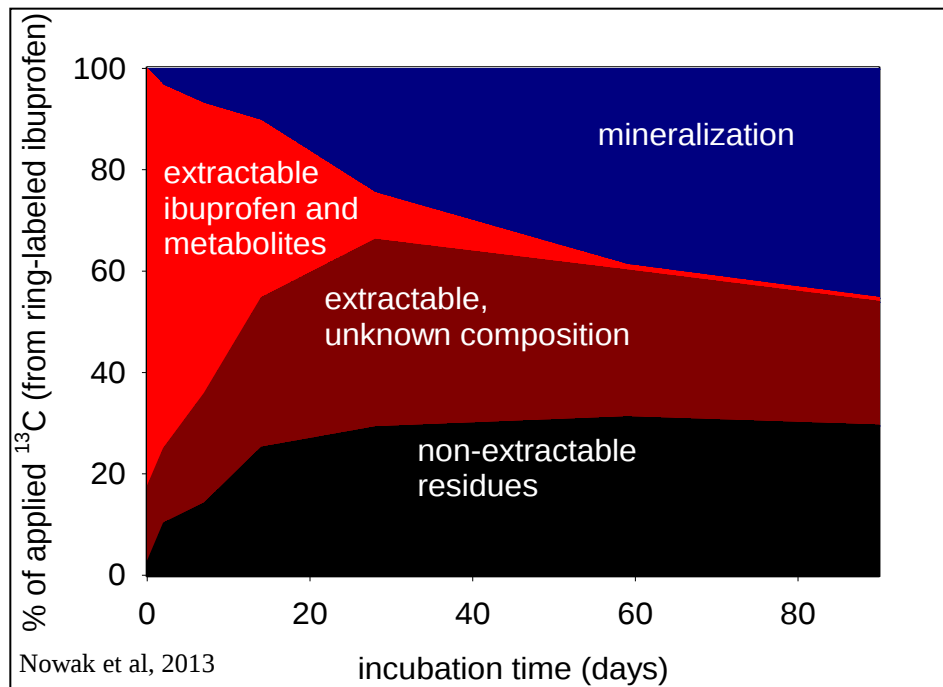
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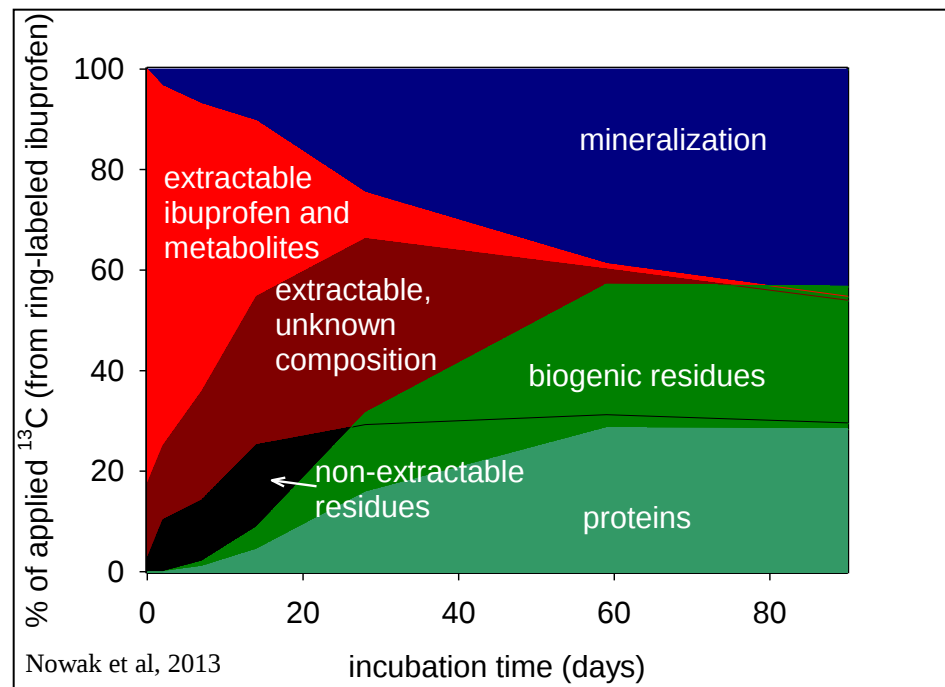
CONCLUSIONS

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General mass balance



New mass balance incl. BioNER



- extractable (unknown composition): bioNER?

Nearly all NER biogenic!

BioNER FROM CO₂ FIXATION

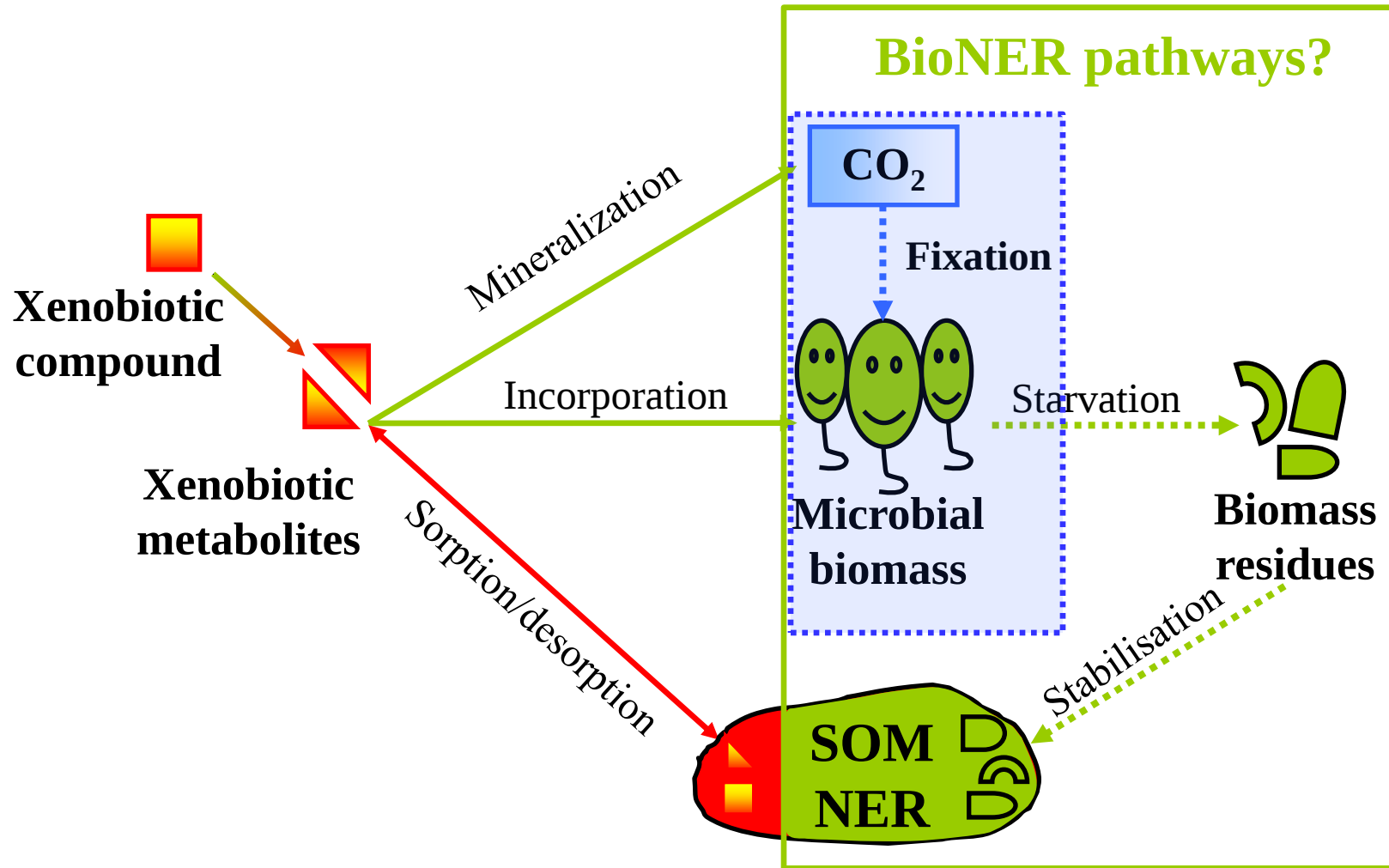
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CO₂ FIXATION EXPERIMENT

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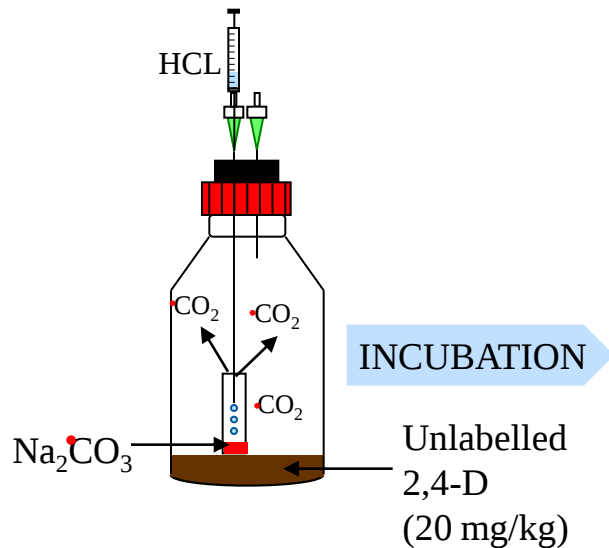
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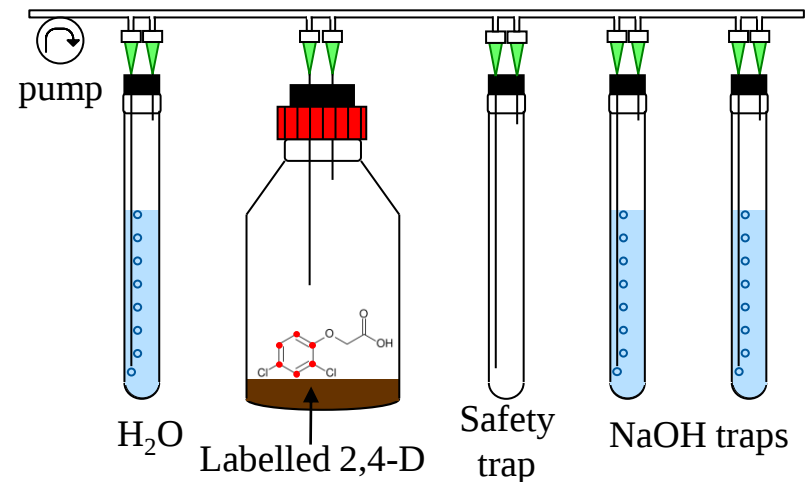
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PLANS...

¹³CO₂ experiment



¹³C₆-2,4-D experiment



¹³C-LABEL ANALYSES

BIONER analyses



• Living biomass:
(PLFA)



GC/MS;
GC-C-IRMS

INCORPORATION OF ^{13}C INTO PLFA

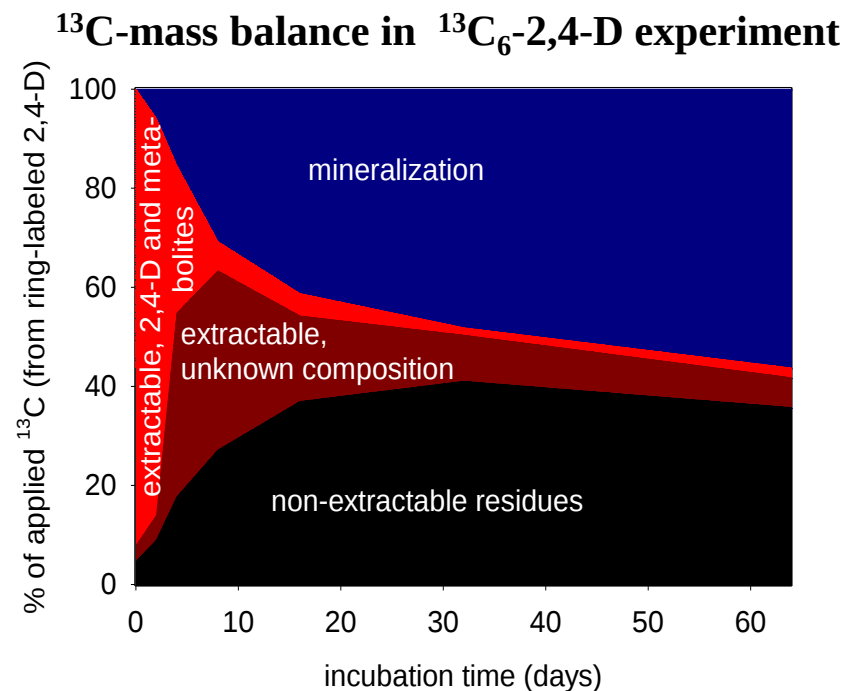
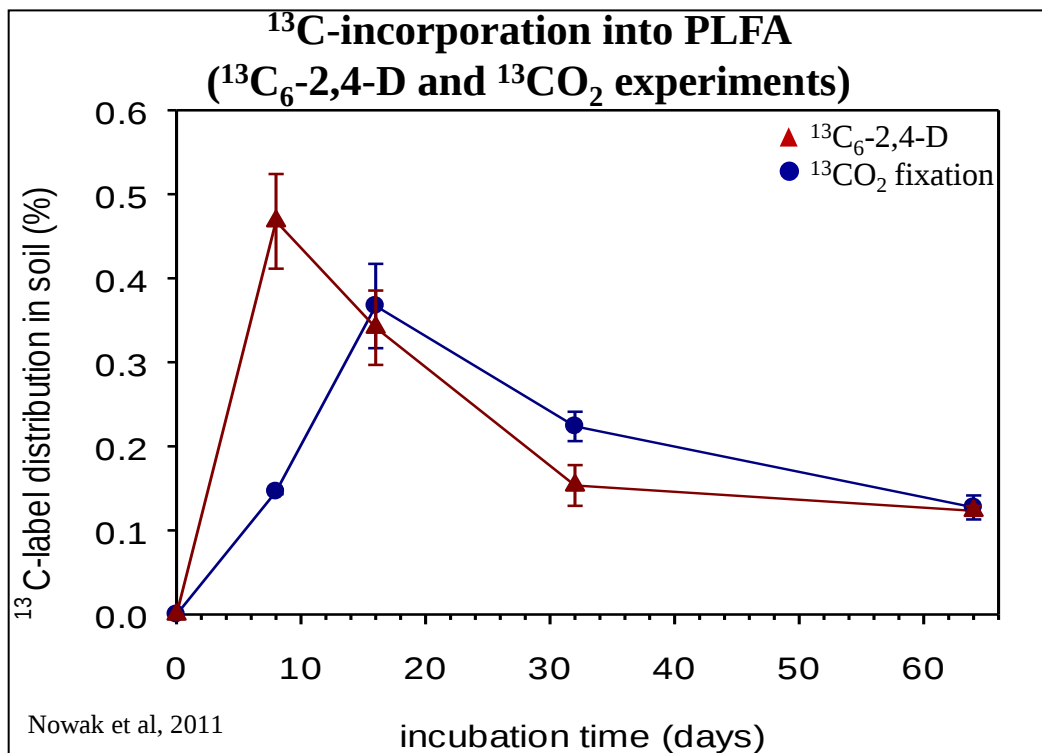
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- $^{13}\text{CO}_2$ fixation (day 16)
- PLFA: decline

^{13}C IN PLFA CLASSES

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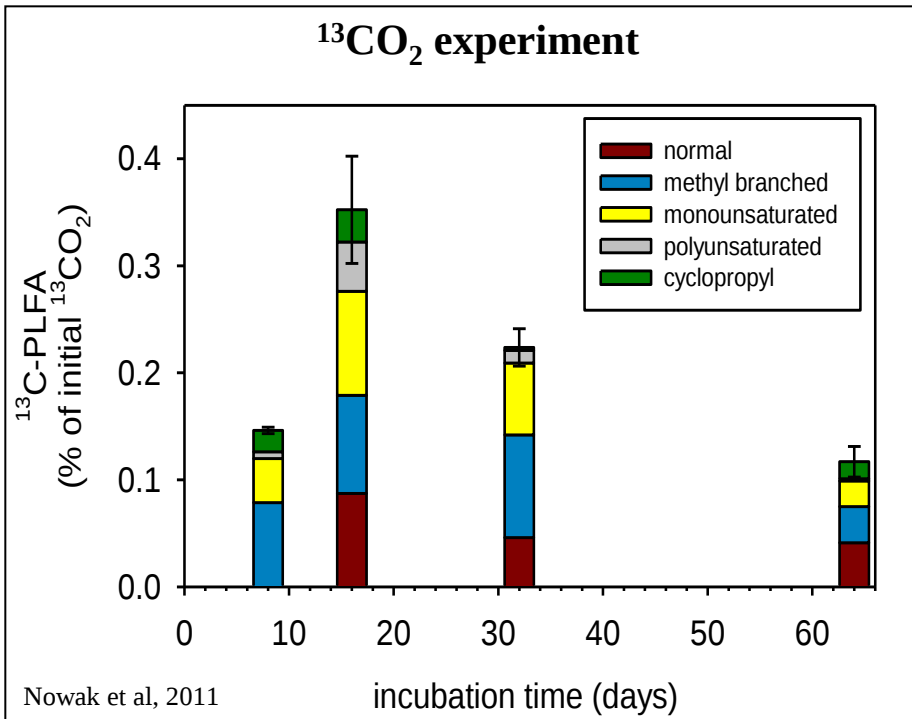
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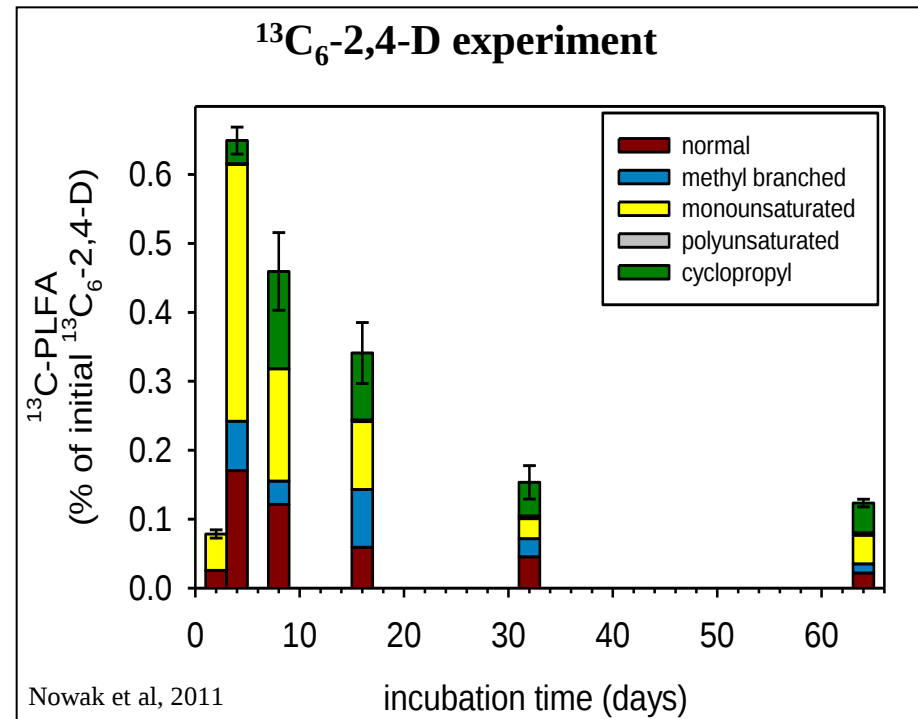
PLANS...

$^{13}\text{CO}_2$ experiment



• G⁺ markers: initially

$^{13}\text{C}_6$ -2,4-D experiment



• G⁻ markers: initially

FINAL REMARKS

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- NER from $^{13}\text{C}_6$ -IBU biogenic = **no risk!**
- NER in abiotic soil low
- tAA high
- bioNER from xenobiotic and CO_2

HOWEVER:

- no biodegradation → xenobiotic NER
- bioNER → biodegradation ($\uparrow\text{CO}_2$) → SOM formation

SOM FORMATION

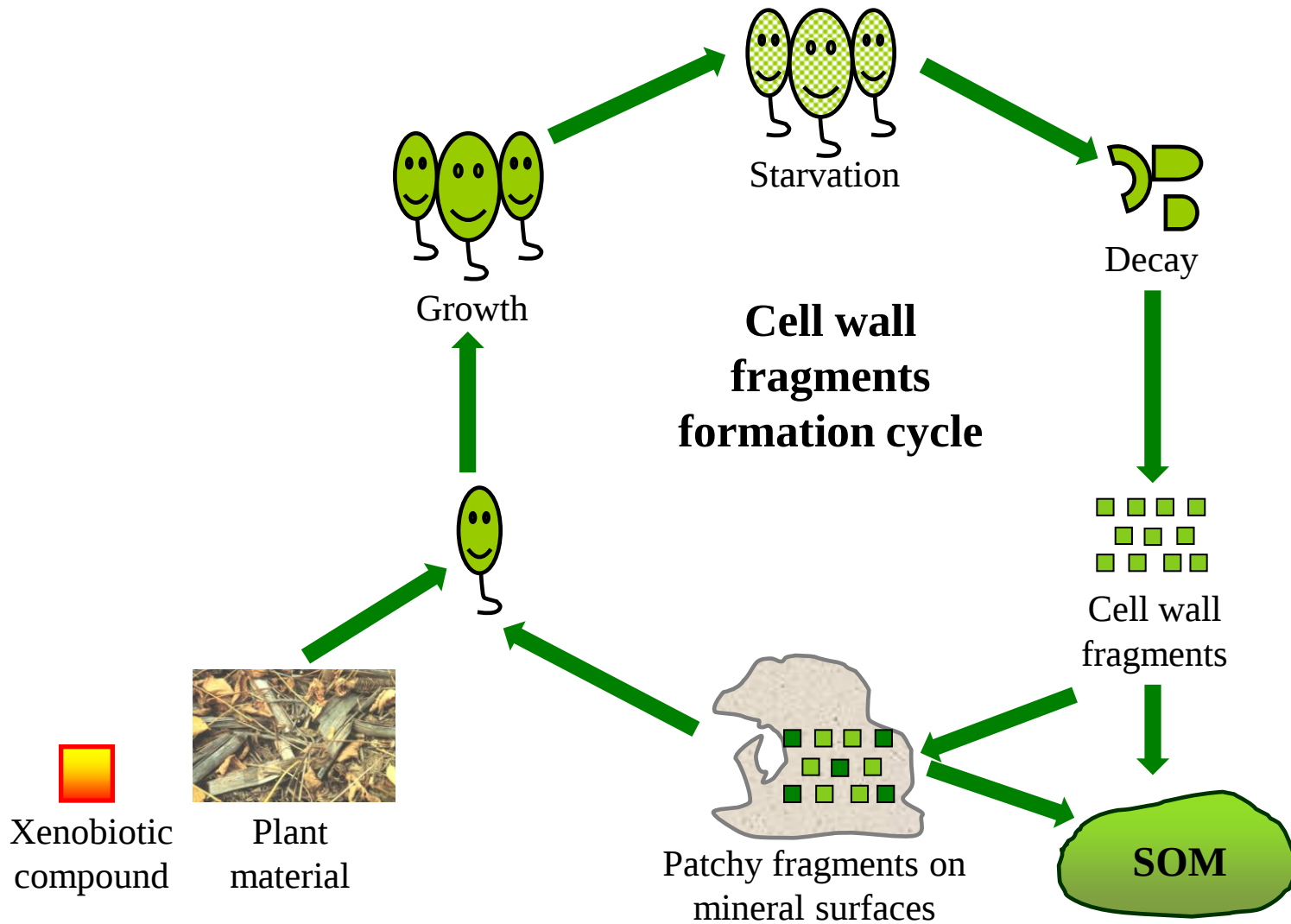
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HOWEVER:

- no biodegradation → xenobiotic NER
- bioNER → biodegradation ($\uparrow\text{CO}_2$) → SOM formation

AND:

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

NER CLASSIFICATION

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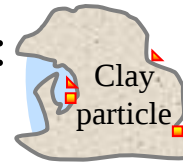
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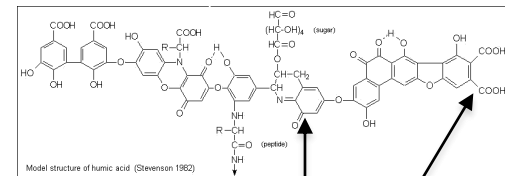
- **type I: sequestered NER:**



- reversible
- remobilisation → risk for the environment

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

- irreversible
- low risk for environment



xenobiotic binding

- **type III: bioNER**



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

ABIOTIC vs BIOTIC NER FORMATION

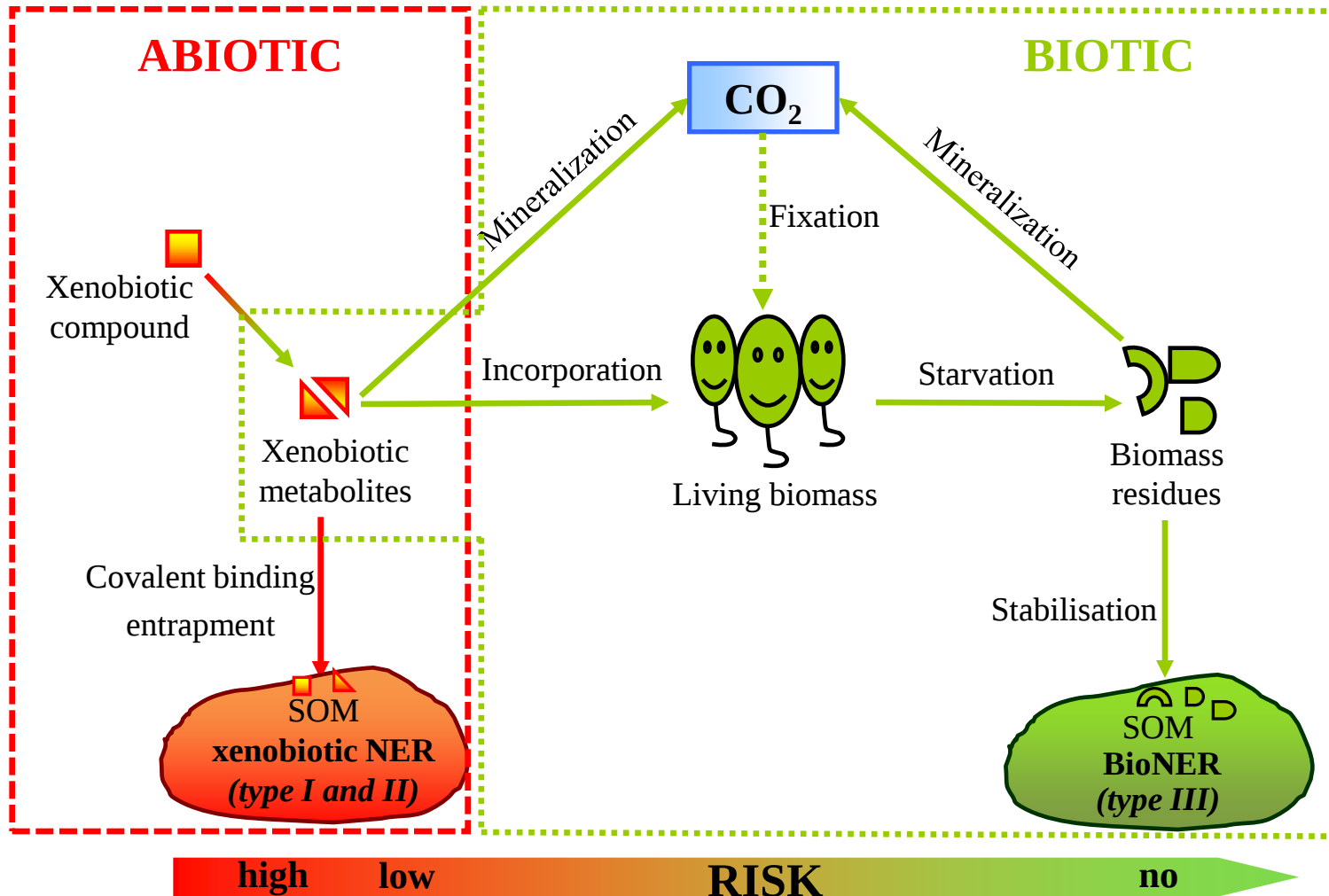
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FURTHER RESEARCH

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- 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/CH₃Cl
(0.8/2/1, v:v)

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

Purification: silica gel ⇒ **PLFA**
(CH₃Cl, 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/Polyethyleneglycol 600
(0.1%/2.5%)

Hydrolysis: 6M HCl, 110°C

Purification: DOWEX 50W-X8
(oxalic acid, 0.01M HCl, H₂O, 2.5M NH₄OH)

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

Purification:
(PB:CH₃Cl)



**GC/MS;
GC-C-IRMS**