



UNIVERSITY OF  
THESSALY



DEPARTMENT OF  
**Biochemistry &  
Biotechnology**  
UNIVERSITY OF THESSALY



H2020 MSCA-IF-2017  
EMIGRATE (grant #749463)

Elucidating the roles and interactions of the members of a bacterial consortium along the degradation of the recalcitrant fungicide thiabendazole via a multi-omic approach

**Sotirios Vasileiadis**

University of Thessaly (**Greece**): Chiara Perruchon, **Dimitrios G. Karpouzas\***

Agricultural Research Institute of **Cyprus**: Michalis Omirou

Helmholtz Centre for Environmental Research GmbH (**Germany**): Benjamin Scheer, Lorenz Adrian, Nicole Steinbach, Antonis Chatzinotas

project site: <http://emigrate.bio.uth.gr>

OMIC  
ENGINE

# Background - Aims

Thiabedazole affects cells:

- fungicide mainly used in post-harvest treatment (inhibits microtubular function)
- Antihelminthic (inhibits fumarate reductase)
- possible effect on bacterial growth has been hypothesized (purine synthesis due to competition?)

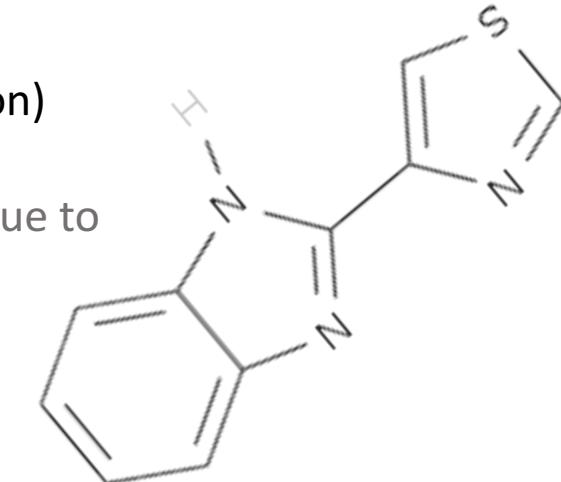
Accumulates in the environment

- recalcitrant under temperate conditions ( $DT_{50} \simeq 403$  days in soil)
- previously, no known biodegraders
- In the absence of economically viable methods, land spreading is a common practice

Recent UTh work:

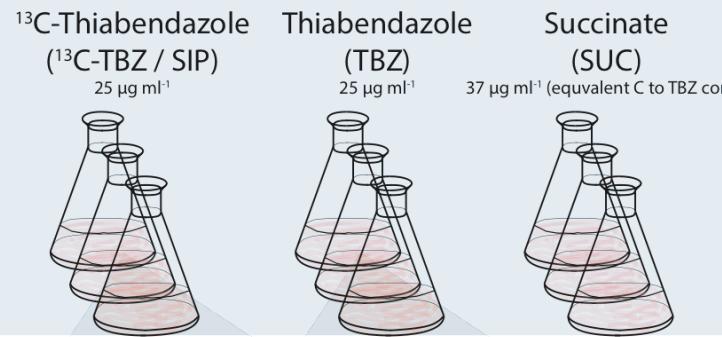
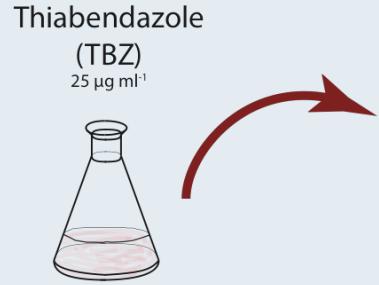
- enrichment of a consortium able to degrade it
- Diversity analysis and a first round of SIP
- antibiotics treatment for isolation -> phenotype loss

**Aims: characterize the consortium and identify the upper pathway enzymes using an omics approach**



# Experimental setup

Minimal salts medium with nitrogen (MSMN) inoculated with a TBZ grown consortium (5%) with sole carbon source:

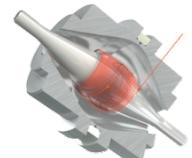


## Bioassays

### TBZ content (HPLC)



### Orbitrap LC-MS



### Time resolved

TBZ screening (HPLC)

16S rRNA gene diversity (HiSeq)

### Time resolved

TBZ degradation (HPLC)

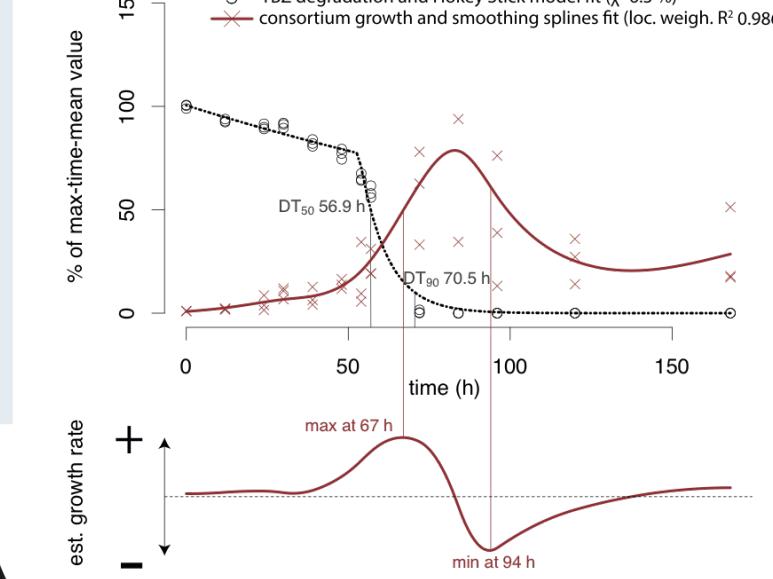
16S rRNA gene quantity (qPCR)

16S rRNA gene diversity (HiSeq)

Shotgun sequencing: DNA (4 conditions) and rRNA depleted RNA (3 timepoints X 2 treat. X 3 reps)

Metabolites (LC/ESI-MS)

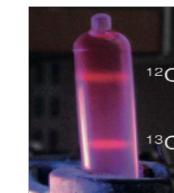
Proteins (orbitrap LC-MS on corresponding RNAseq samples)



stable isotope probing (SIP)  
mass based DNA fractionation



Neufeld et al, 2007  
doi:10.1038/nprot.2007.109



### 16S rRNA gene copies (qPCR)



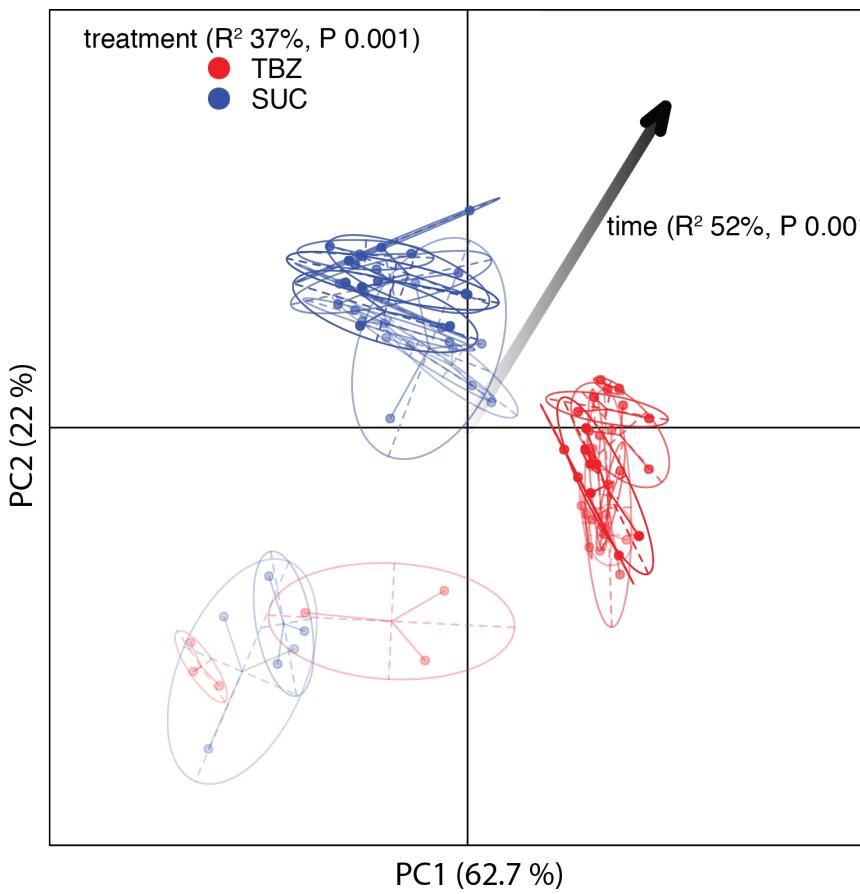
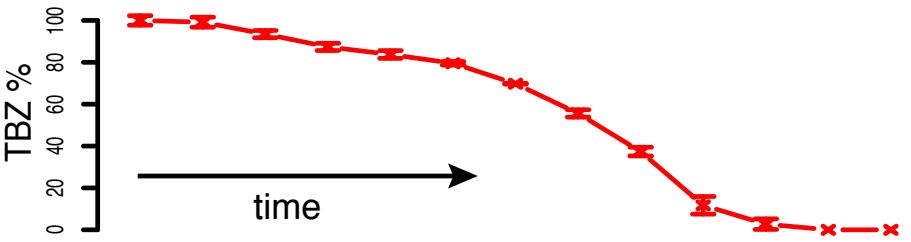
### Metabolites (LC/ESI-MS)



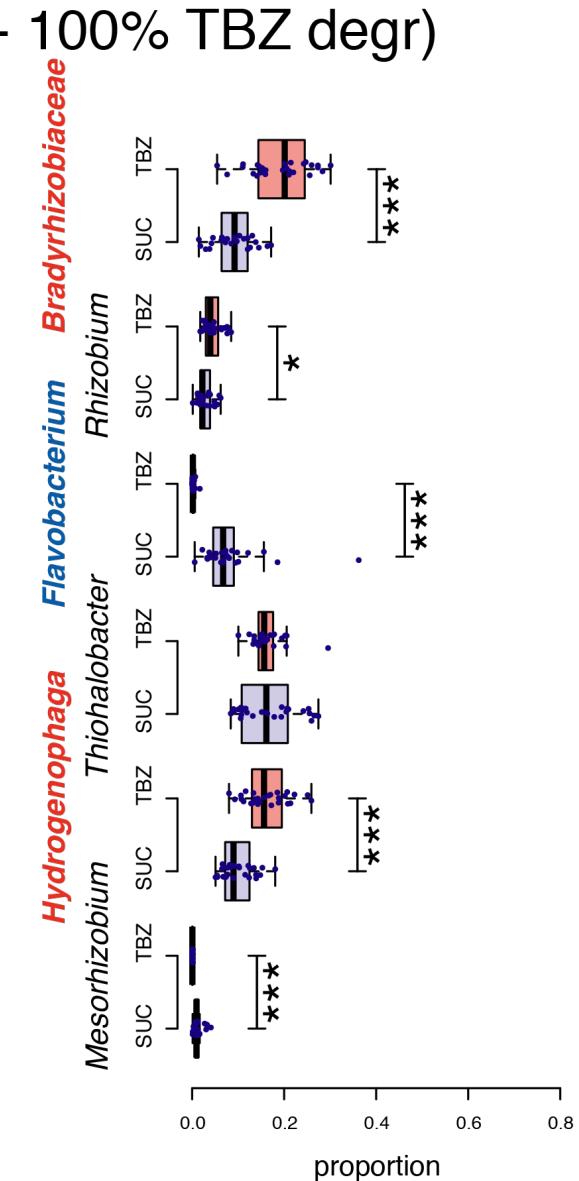
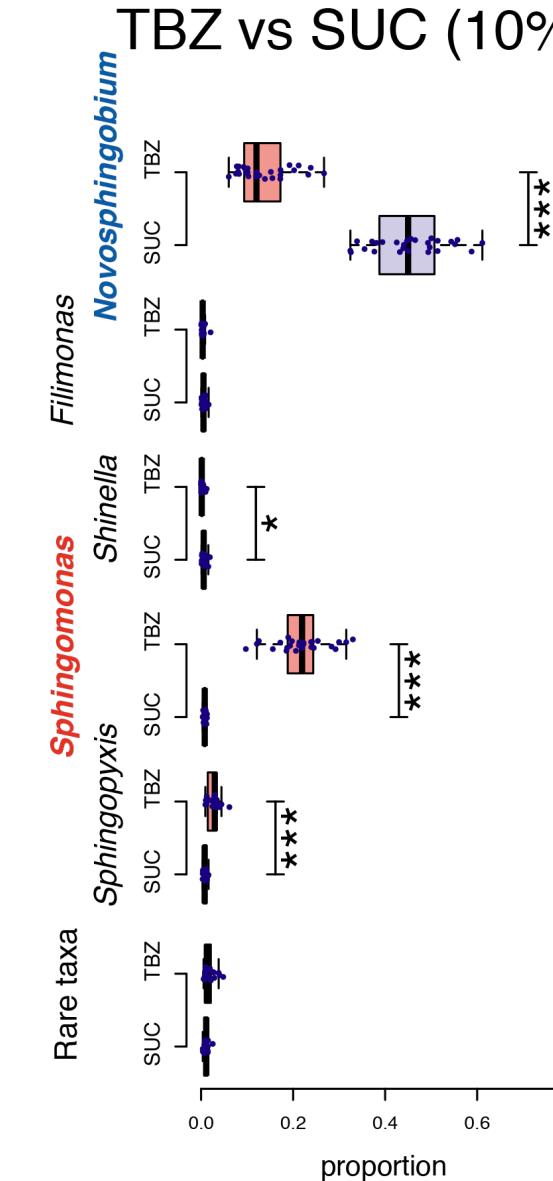
Illumina HiSeq (2X250) sequencing  
16S rRNA gene V4  
shotgun DNA/RNA



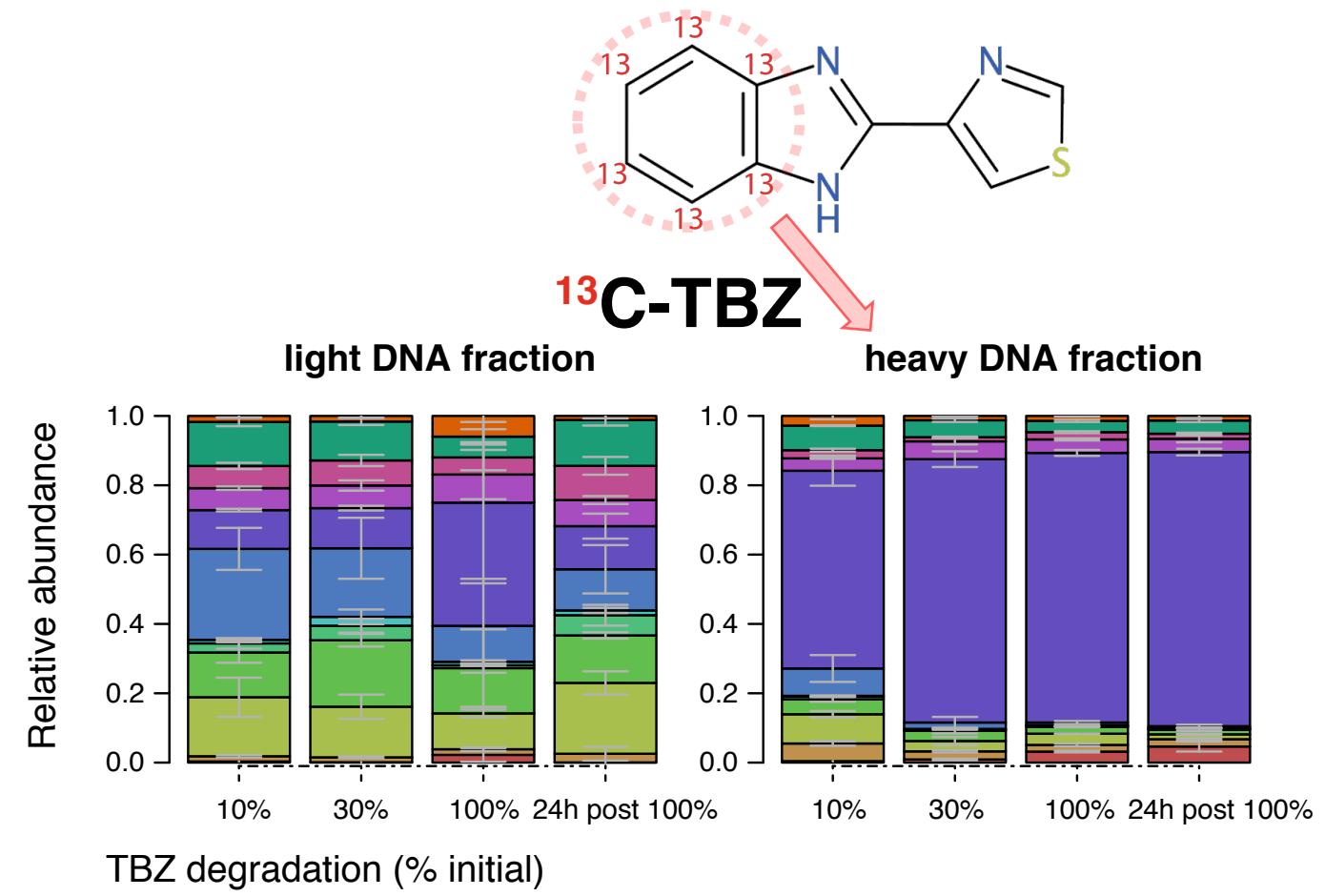
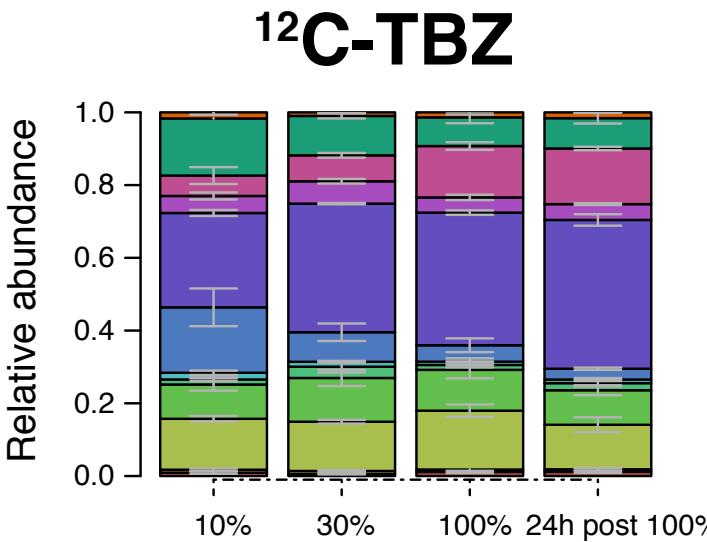
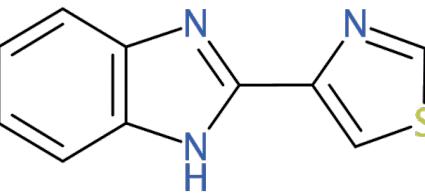
# SSU diversity



TBZ vs SUC (10% - 100% TBZ degr)



# SIP (<sup>13</sup>C) SSU diversity



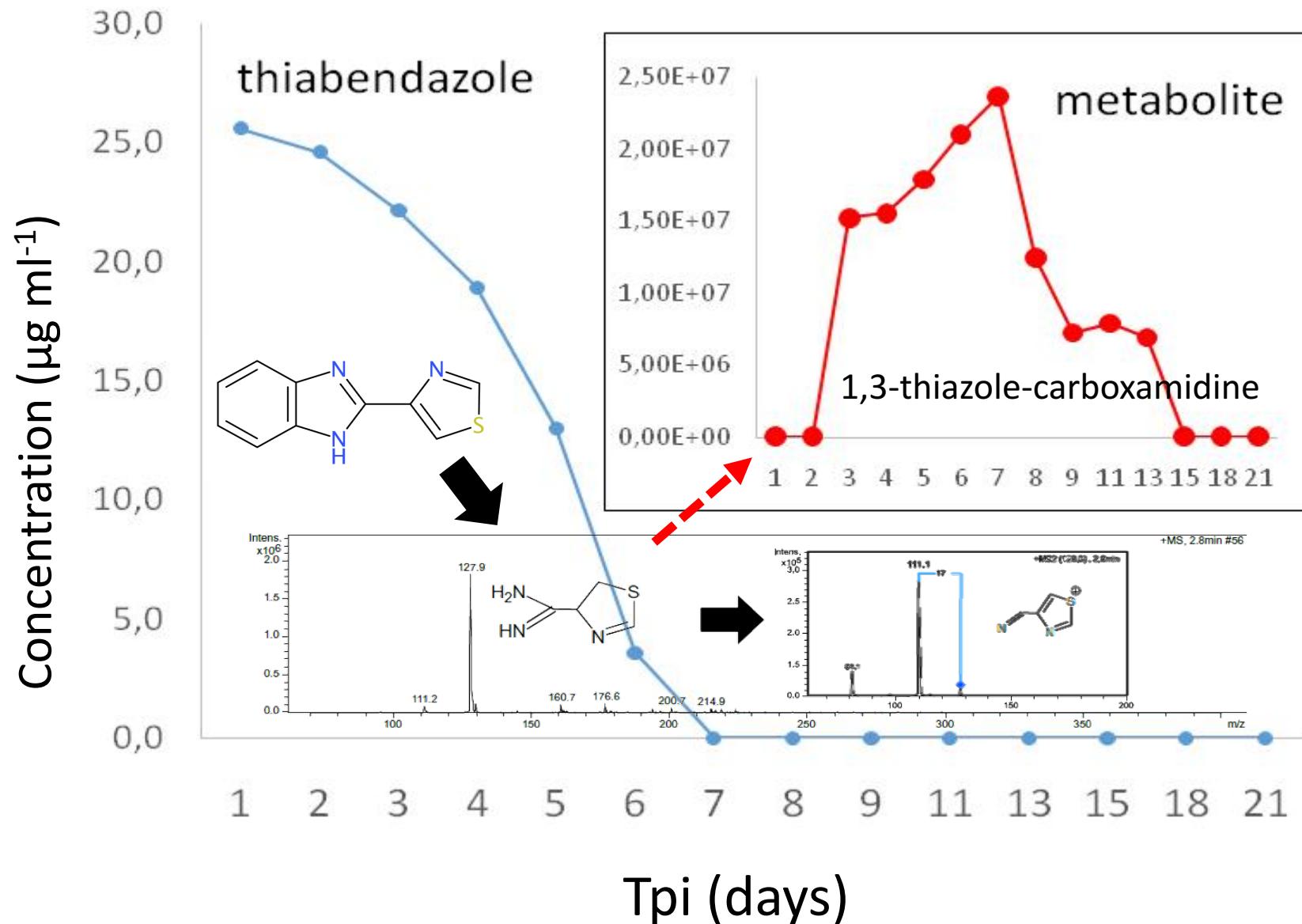
■ *Microbacterium*  
■ *Pedobacter*  
■ *Afipia*

■ *Bradyrhizobium*  
■ *Hyphomicrobium*  
■ *Shinella*

■ *Novosphingobium*  
■ *Sphingomonas*  
■ *Hydrogenophaga*

■ *Thiobacillus*  
■ *Hydrocarboniphaga*  
■ < 2 % relative abund.

# TBZ main metabolite



# consortium metagenome

Metawatt (Strous et al 2012; 10.3389/fmicb.2012.00410) bin IDs and stats		MiGA (Rodriguez-R et al, 2018; 10.1093/nar/gky467) NCBI hits			MiGA binning quality			
Bin ID	cntag# // size(nt) // N50(nt) // GC(%)	Class	Strain	AAI or ANI (%)	MiGA classification confident at level	completeness (%)	contamination (%)	quality (%)
Bin 3X21F	38 // 3,388,361 // 206,417 // 62.4	α-Proteobacteria	<i>Sphingomonas</i> sp. DC 6	61.91 (AAI)	Genus	95.5	0.9	91
Bin 34A	52 // 4,618,291 // 136,635 // 65.2	γ-Proteobacteria	<i>Thiohalobacter thiocyanaticus</i>	45.72 (AAI)	Class	94.6	0.9	90.1
Bin 19A	189 // 5,487,813 // 61,470 // 65.8	β-Proteobacteria	<i>Hydrogenophaga</i> sp. RAC07	78.96 (AAI)	Subspecies	90.1	1.8	81.1
Bin 9B	25 // 4,220,254 // 315,044 // 62.1	α-Proteobacteria	<i>Bradyrhizobiaceae bacterium SG 6C</i>	92.33 (ANI)	Subspecies	94.6	0.9	90.1
Bin 13A	118 // 5,825,532 // 101,074 // 67.8	β-Proteobacteria	<i>Hydrogenophaga</i> sp. RAC07	81.07 (AAI)	Subspecies	91.9	6.3	60.4
Bin 23F	283 // 5,140,160 // 107,438 // 69.6	β-Proteobacteria	<i>Hydrogenophaga</i> sp. PBC	89.76 (AAI)	Subspecies	89.2	2.7	75.7

13 low abundance bins	3,862 // 61,120,920 // 101,474
<100Kb bins	571 // 1,464,056 // 4,814
unbined	1,650 // 7,278,568 // 24,154
Total	6,788 // 98,543,955 // 100,895

Bin relative abundance				
	13C TBZ 10% degradation	50% TBZ degradation	100% TBZ degradation	24 h post TBZ disappearance
<i>Sphingomonas</i> sp. DC 6	64.76	23.96	19.62	10.05
<i>Thiohalobacter thiocyanaticus</i>	1.11	13.48	22.78	29.17
<i>Hydrogenophaga</i> sp. RAC07	0	11.71	1.83	0
<i>Bradyrhizobiaceae bacterium SG 6C</i>	2.58	5.93	17.83	25.23
<i>Hydrogenophaga</i> sp. RAC07	0.05	5.18	2.24	2.06
<i>Hydrogenophaga</i> sp. PBC	1.29	4.21	10.02	12.35

1% < x < 5%	
5% < x < 10%	
10% < x	

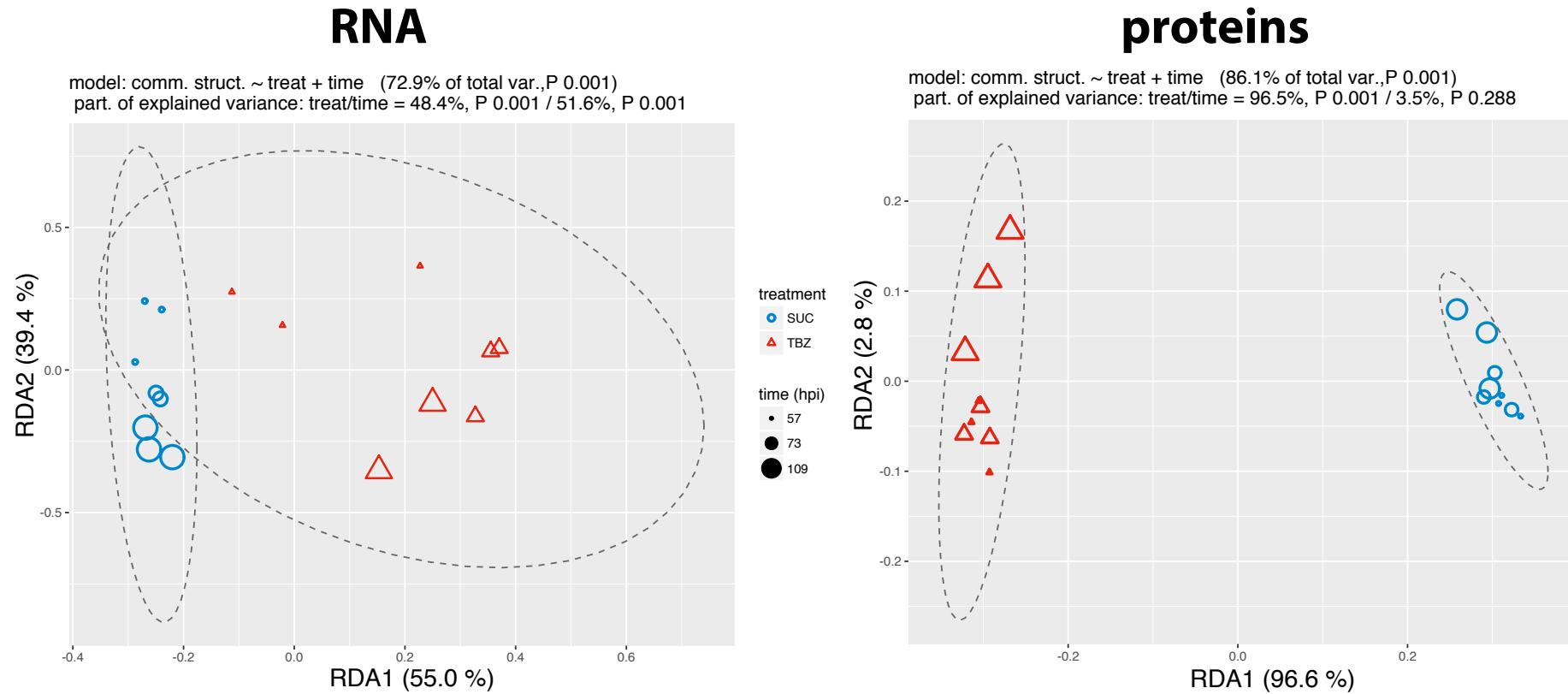
# Gene expression profile

(40% degradation, 100% degradation, 24h post degradation)

TBZ/SUC

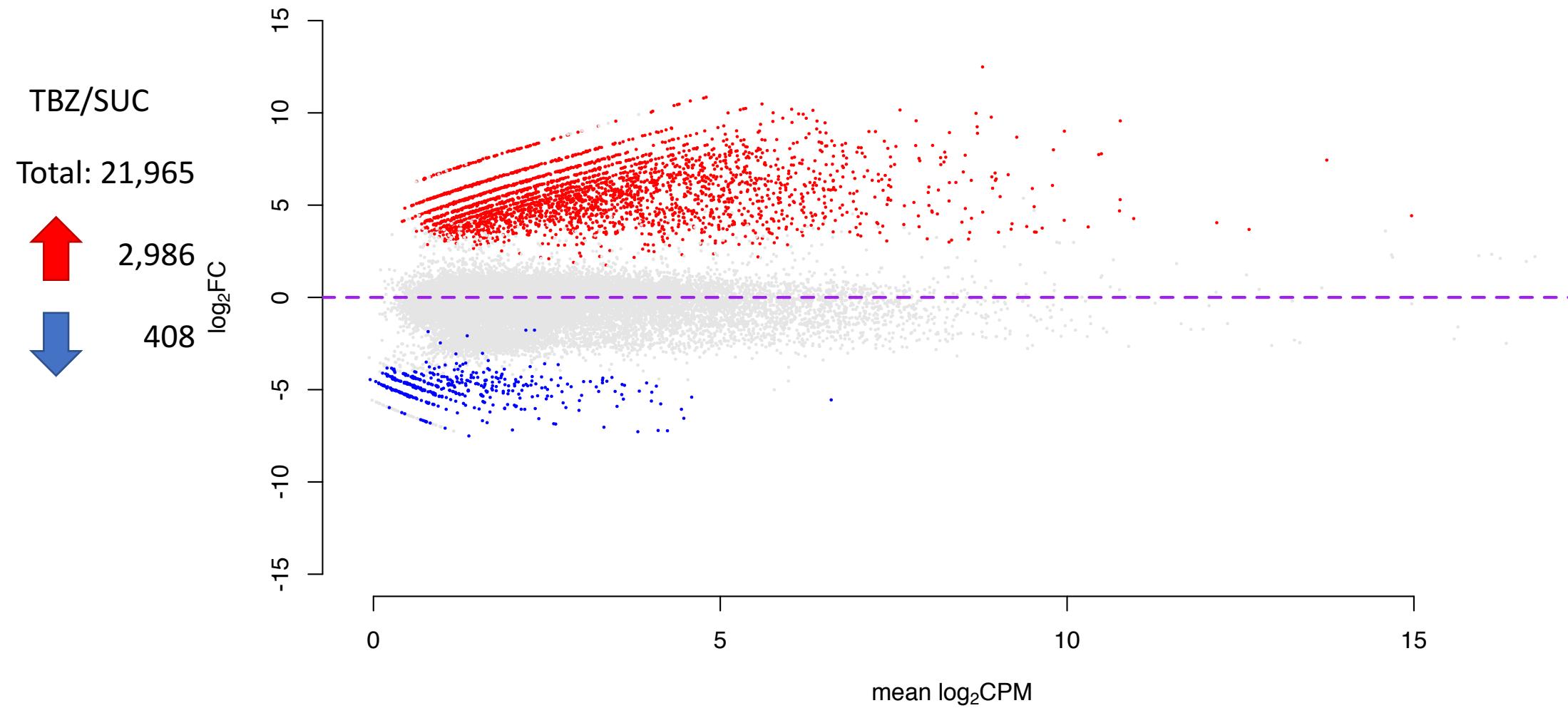
Total: 21,965

↑ 2,986  
↓ 408



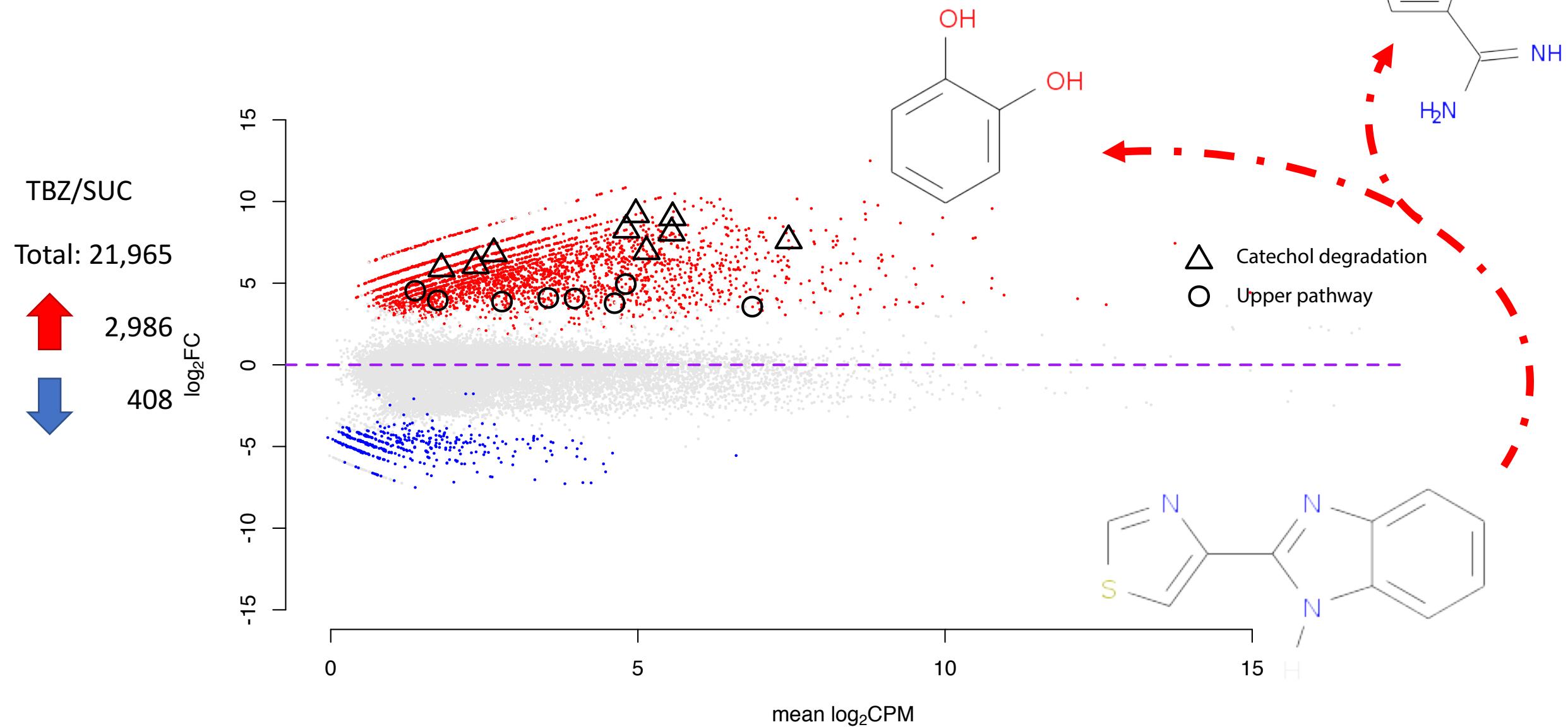
# Gene expression profile

(Putative degradation genes)



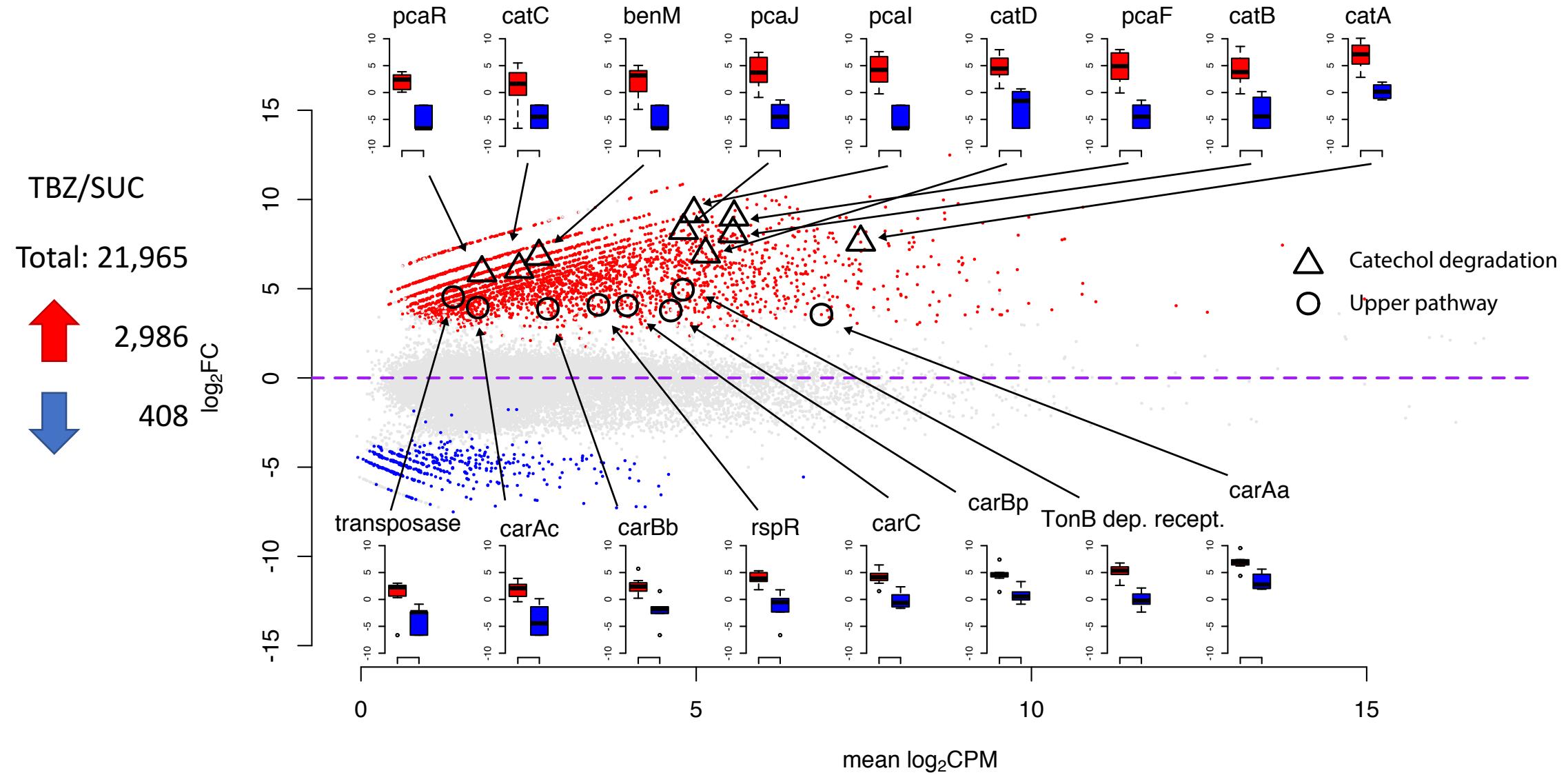
# Gene expression profile

(Putative degradation genes)



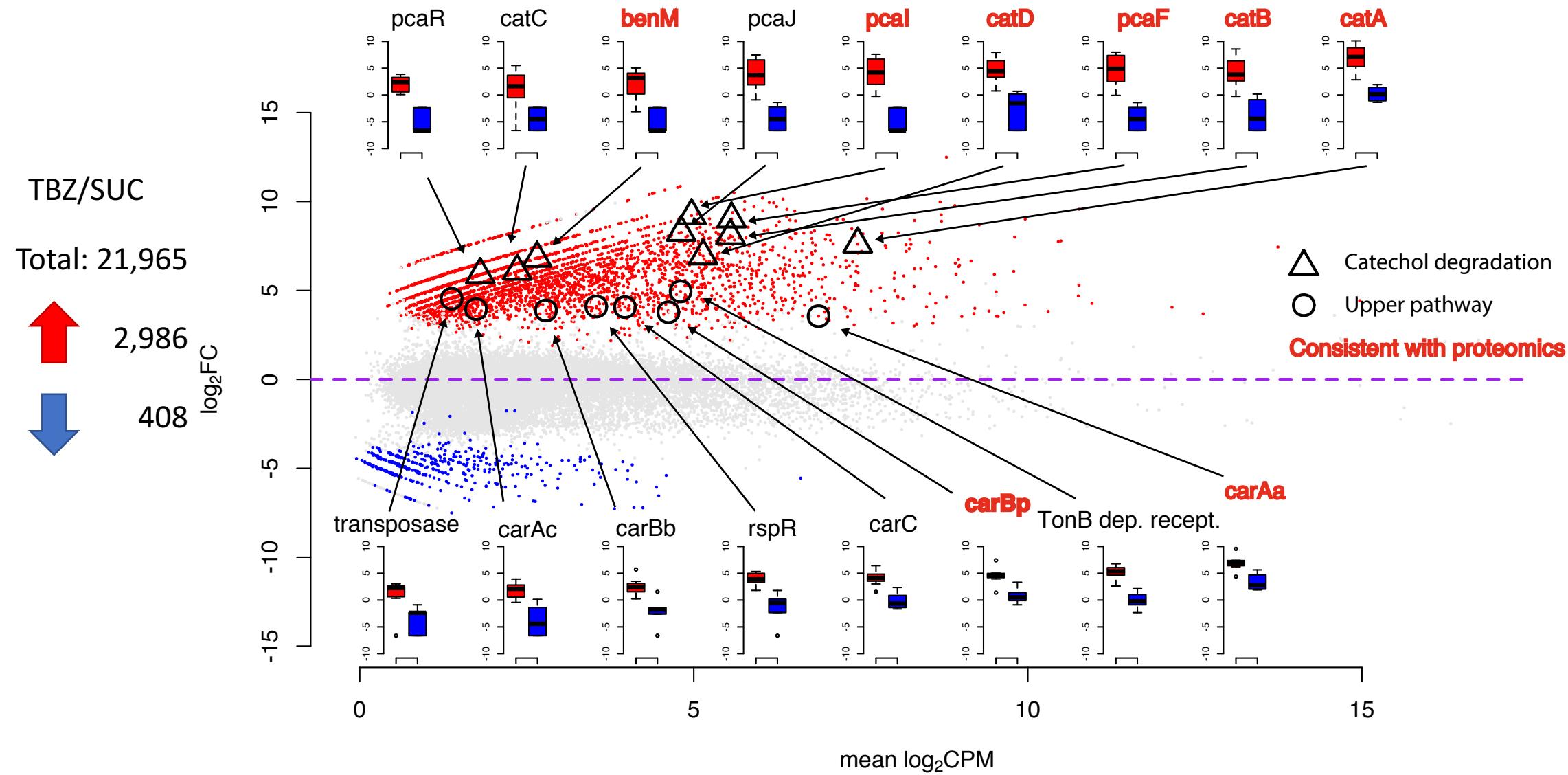
# Gene expression profile

## (Putative degradation genes)



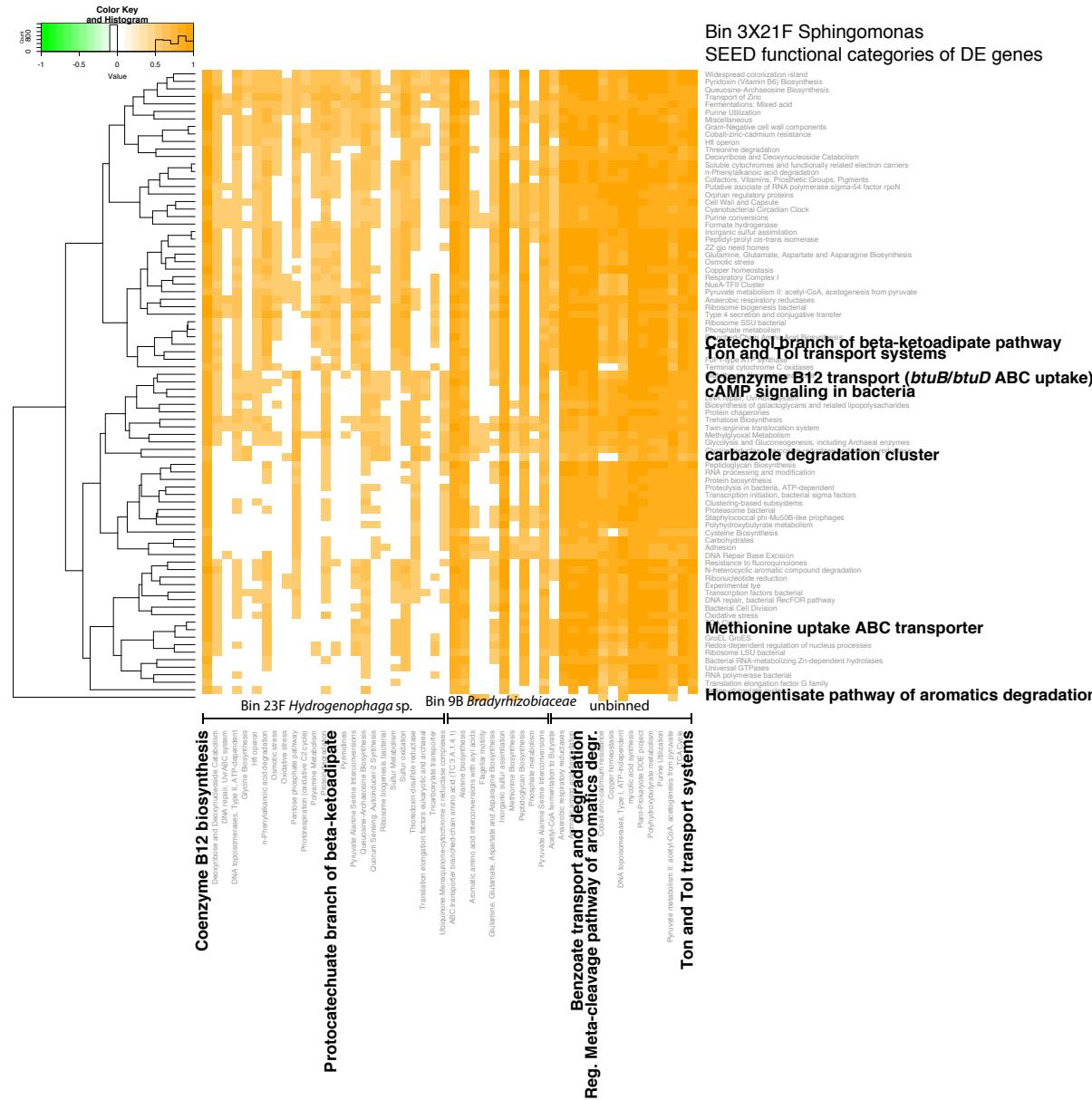
# Gene expression profile

## (Putative degradation genes)

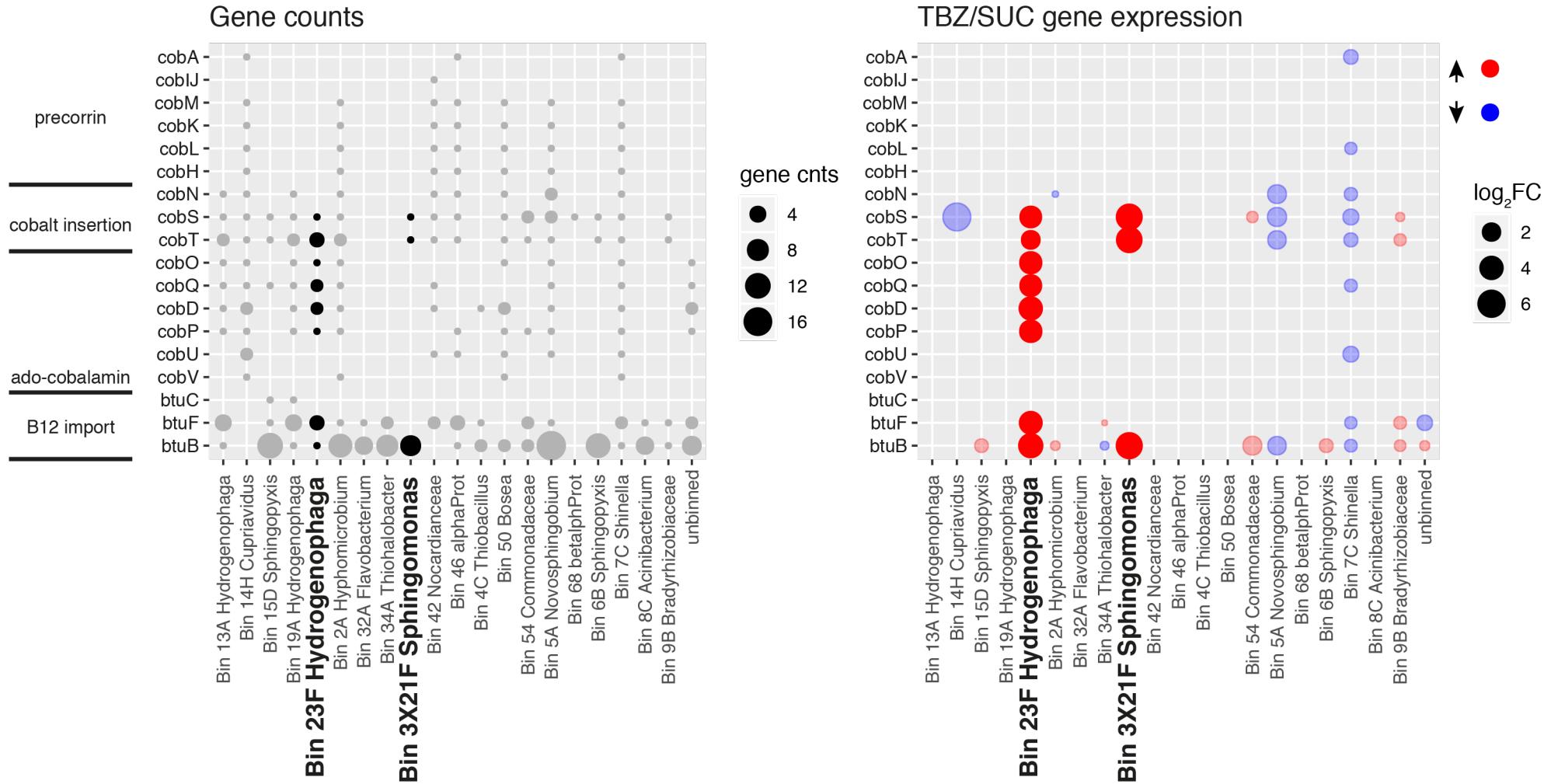


# Gene expression profile

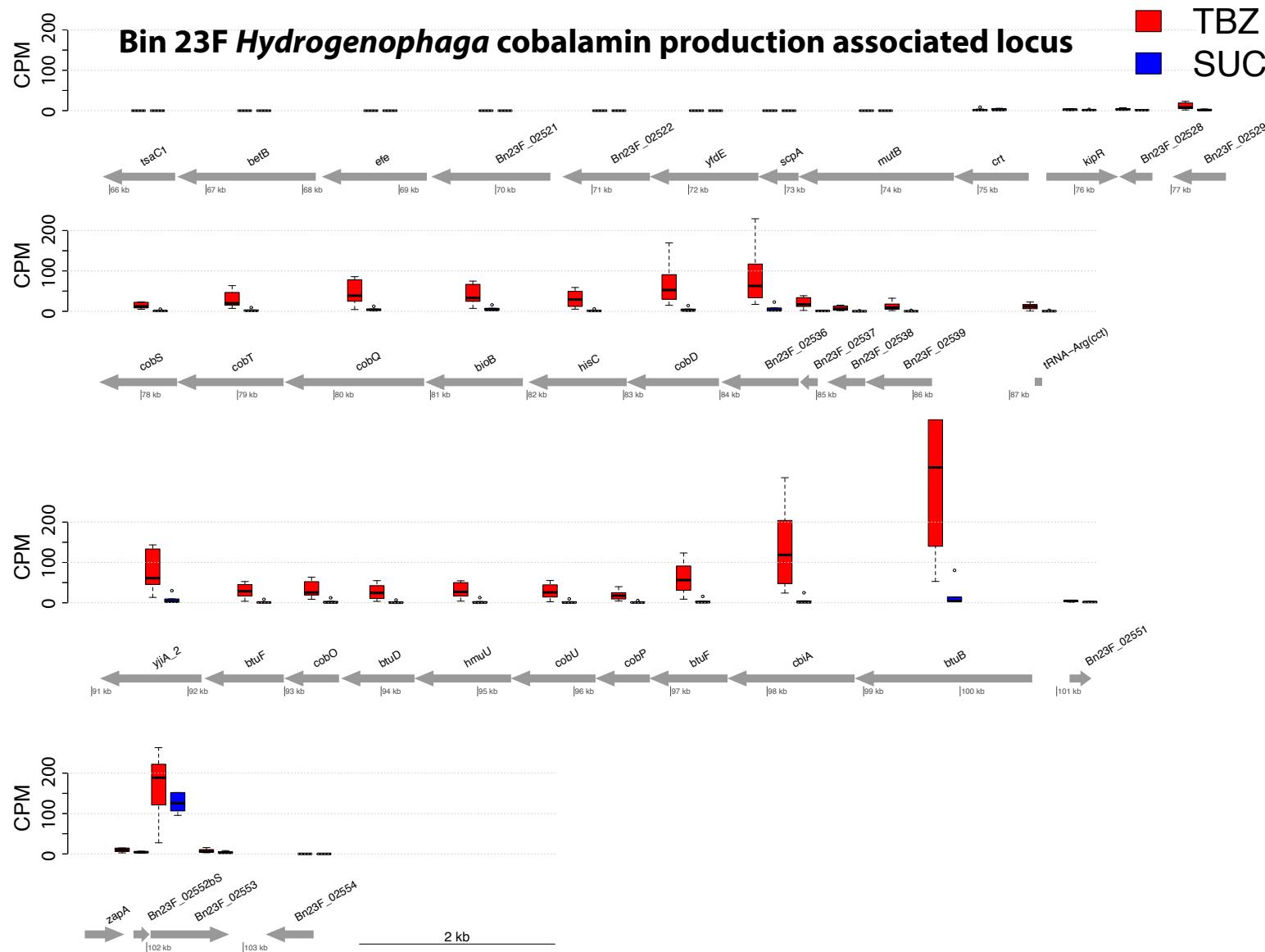
## (network analysis – *Sphingomonas* subcluster)



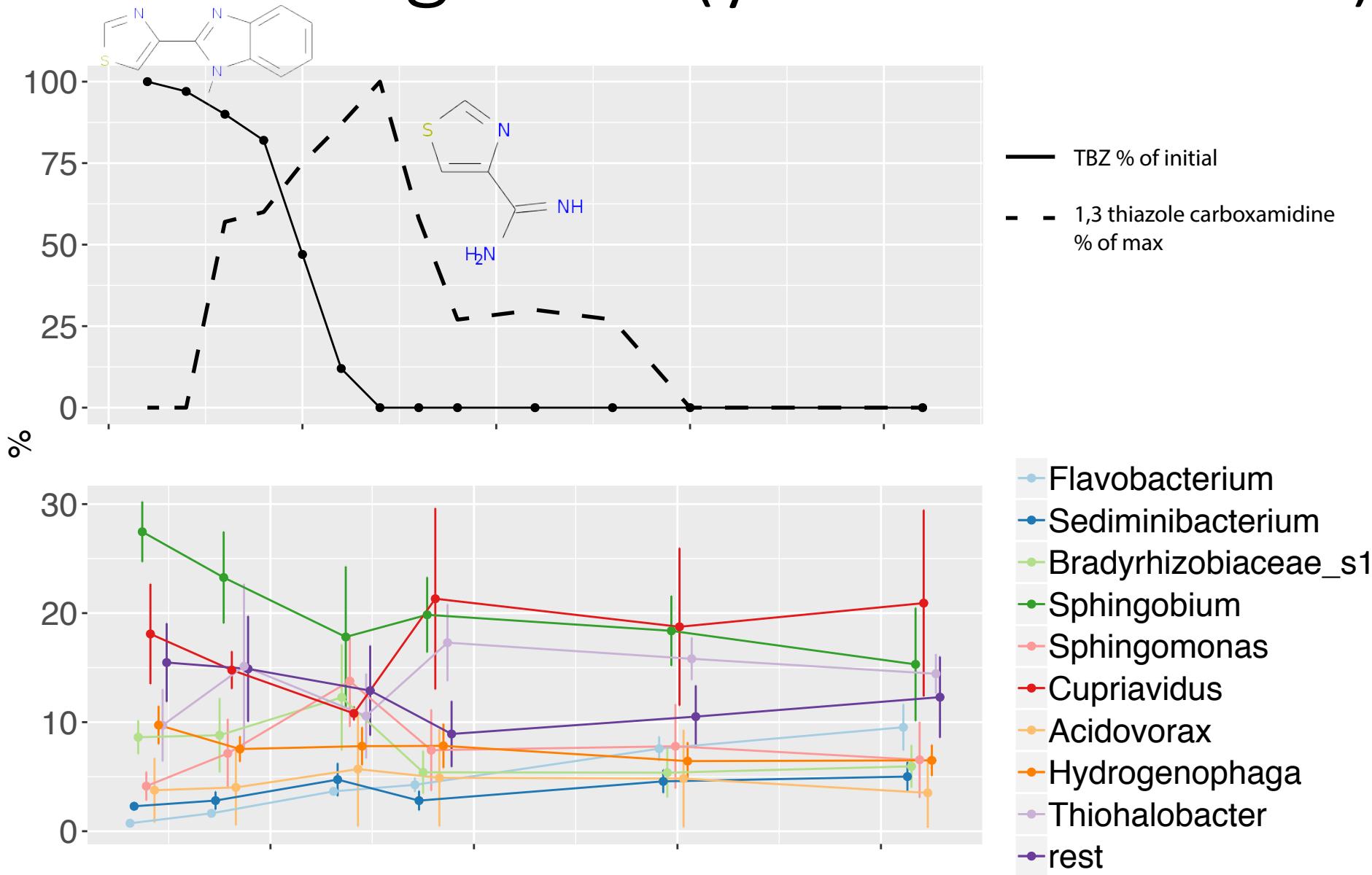
# Cobalamin pathway completeness/expression



# Gene expression profile



# TP degrader (yet inconclusive)



# Concluding remarks

- ✓ ***Sphingomonas* carries out the upper TBZ degradation pathway**
- ✓ **1,3 thiazole dicarboxamidine is the main TP**
- ✓ **Cobalamin-associated auxotrophy possibly regulates the growth of the major degrader**
- ✓ **The consortium is structurally stable along TBZ degradation**
- ✓ **SIP, shotgun metagenomics and RNAseq provided a wealth of valuable information**

... to do ...

- ✓ **Candidate strain putative genomes are being examined for TP degradation**
- ✓ **Hard evidence retrieval is on the way!**



UFZ Environmental Microbiology

Antonis Chatzinotas  
Nicole Steinbach



UFZ Isotope Biochemistry

Lorenz Adrian  
Benjamin Scheer



Agr. Res. Inst. Of Cyprus  
Michalis Omirou

Thank you!!!



Dimitrios G Karpouzas  
Chiara Perruchon

