Interactomics of the degradation of a recalcitrant pesticide by a soil-enriched bacterial consortium

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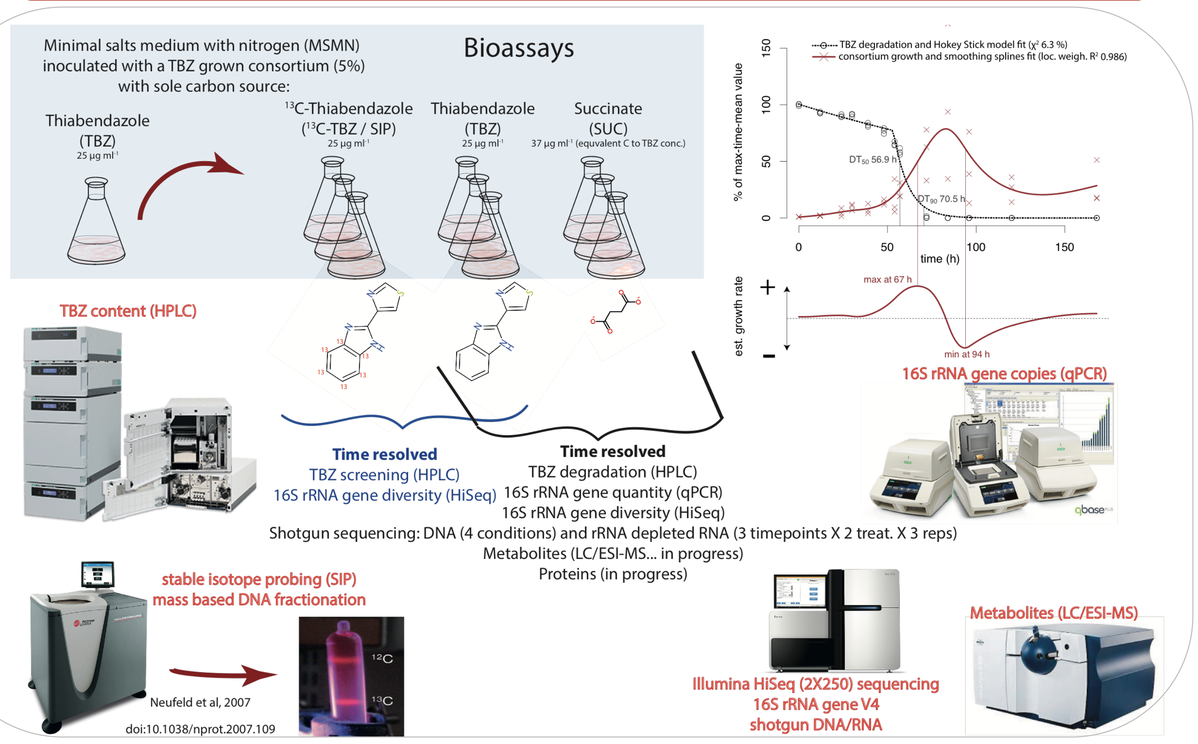
***Funding: H2020 MSCA-IF-2017 project EMIGRATE (grant #749463)**

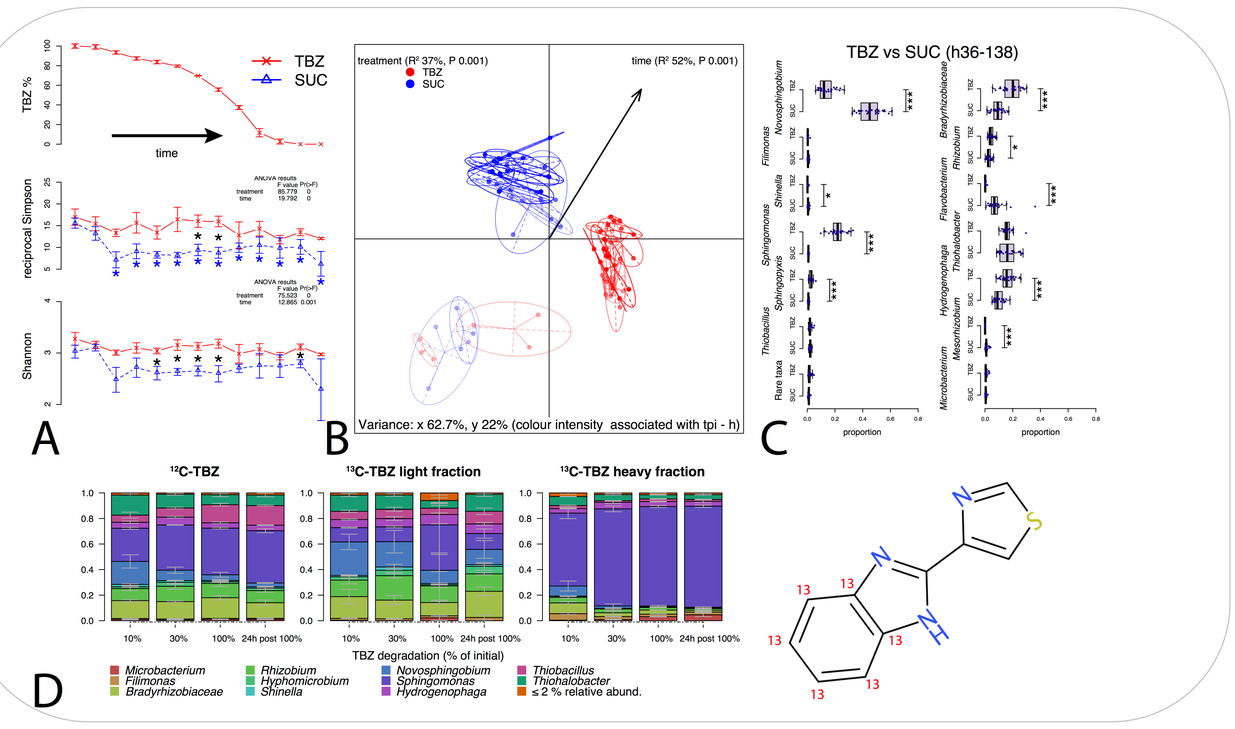
Background

Recalcitrant to degradation compounds used in agriculture pose a challenge for environmental management. Thiabendazole (TBZ), a benzimidazole commonly used protectively against postharvest fungal diseases and as anthelminthic in livestock farming, is highly stable (DT_{50} > 1-2 years) in the environment without previously known potent biodegraders. A recently soil-enriched bacterial consortium able to rapidly degrade TBZ, resulted in no TBZ-degrading isolate, suggesting complex interactions between members. We employed an "omics" approach to elucidate the microbial interactions that maintain the degradation consortium capability.

Approach



Diversity and SIP analysis



TBZ vs SUC as sole C-source:

A. Major differences at the Shannon index (i.e. higher diversity of low abundance members with TBZ) and less differences at the inverse Simpson (reflecting more dominant members)

B. Distinct community structures, yet, stable beyond 10% TBZ degradation

C. *Sphingomonas*, *Hydrogenophaga*, *Sphingopyxis*, *Bradyrhizobiaceae* higher under TBZ

^{13}C *vs* ^{12}C TBZ as sole C-source:

D. ***Sphingomonas*** is the major ^{13}C-labeled ring assimilator

Meta – genomics/bolomics/transcriptomics

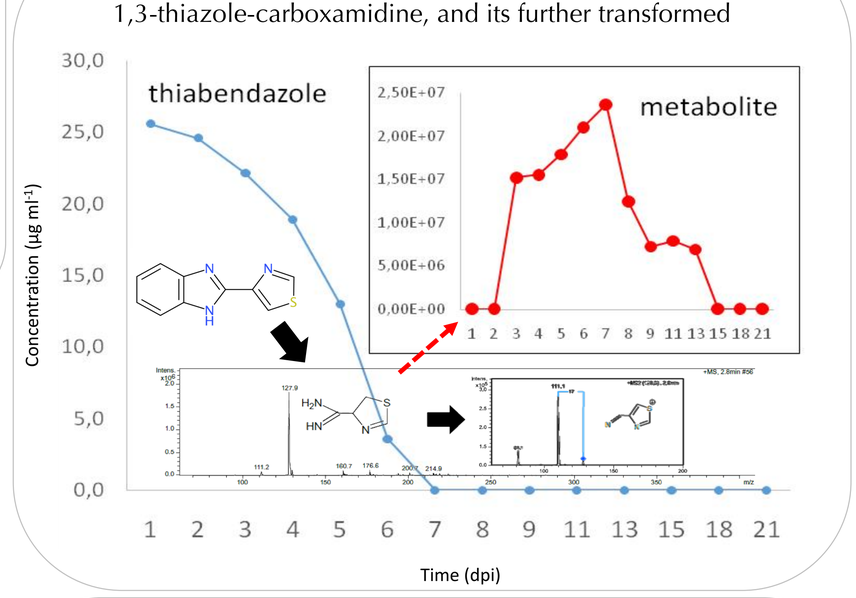
A. Metagenome assembly and binning resulted in six dominant bins (≥1% RA) and 13 less abundant of high/excellent (according to MiGA) quality genomes (more than 80% completeness), with closest hits with mainly oligotrophic/autotrophic lifestyles and also bioremediation potential.

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 relative abundance			
Bin ID	**contigs (#)**	**size (nt)**	**N50 (nt)**	**GC (%)**	**Class**	**Family**	**Strain**	**AAI or ANI (%)**	**MiGA classification confident at level**	**completeness (%)**	**contamination (%)**	**quality (%)**	**13C TBZ 10% degradtion**	**50% TBZ degradation**	**100% TBZ degradation**	**24 h post TBZ dissapearance**
Bin 3X21F	38	3,388,361	206,417	62.44	α-*Proteobacteria*	*Sphingomonadaceae*	*Sphingomonas* sp. DC 6 NZ CP021181	61.91 (AAI)	Genus	95.5	0.9	91	64.76	23.96	19.62	10.05
Bin 34A	52	4,618,291	136,635	65.21	γ-*Proteobacteria*	*Ectothiorhodospiraceae*	*Thiohalobacter thiocyanaticus* NZ AP018052	45.72 (AAI)	Class	94.6	0.9	90.1	1.11	13.48	22.78	29.17
Bin 19A	189	5,487,813	61,470	65.83	β-*Proteobacteria*	*Comamonadaceae*	*Hydrogenophaga* sp. RAC07	78.96 (AAI)	Subspecies	90.1	1.8	81.1	0	11.71	1.83	0
Bin 9B	25	4,220,254	315,044	62.13	α-*Proteobacteria*	*Bradyrhizobiaceae*	*Bradyrhizobiaceae* bacterium SG 6C NZ CM001195	92.33 (ANI)	Subspecies	94.6	0.9	90.1	2.58	5.93	17.83	25.23
Bin 13A	118	5,825,532	101,074	67.84	β-*Proteobacteria*	*Comamonadaceae*	*Hydrogenophaga* sp. RAC07	81.07 (AAI)	Subspecies	91.9	6.3	60.4	0.05	5.18	2.24	2.06
Bin 23F	283	5,140,160	107,438	69.57	β-*Proteobacteria*	*Comamonadaceae*	*Hydrogenophaga* sp. PBC NZ CP017311	89.76 (AAI)	Subspecies	89.2	2.7	75.7	1.29	4.21	10.02	12.35
13 low abundance bins	3862	61,120,920	101,474													
<100Kb bins	571	1,464,056	4,814													
unbined	1650	7,278,568	24,154													
Total	**6788**	**98,543,955**	**100,895**													

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

Sphingomonas bin dominated the initial TBZ degradation stages (phenol ring removal)

B. Metabolome: The main transformation product is 1,3-thiazole-carboxamidine, and its further transformed



Metatrascriptome

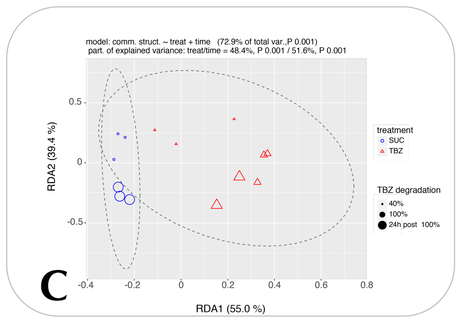
C. Metagenome-wide gene expression patterns show strong structural differences between the TBZ and the SUC treatments and also between the initial TBZ degradation stages and the post TBZ degradation stages

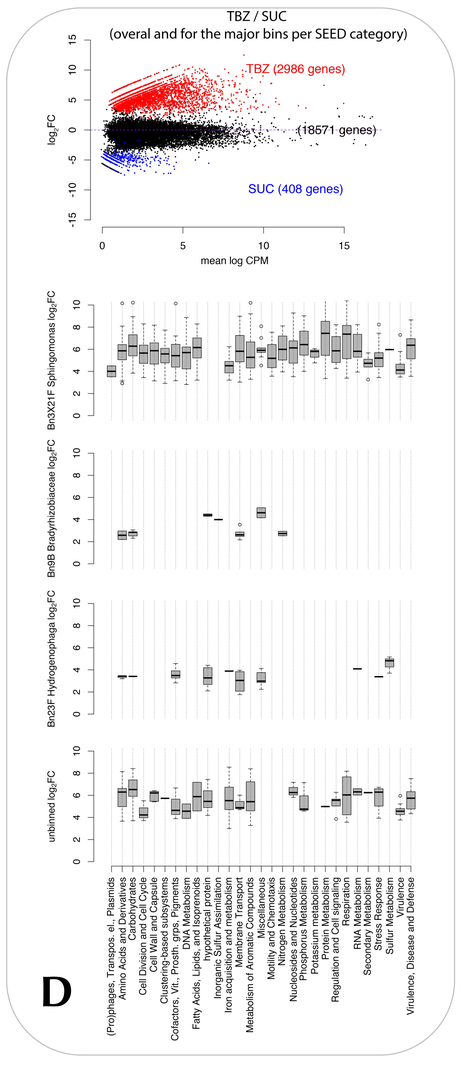
D. Overall and per bin gene-expression/treatment associations at gene and SEED functional category level

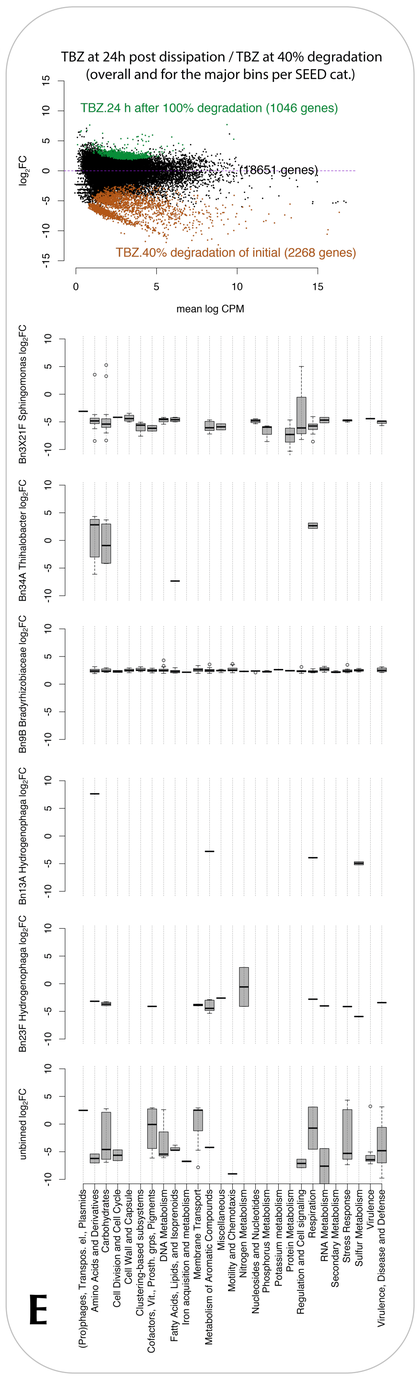
E. Same as D between initial and post TBZ degradation stages

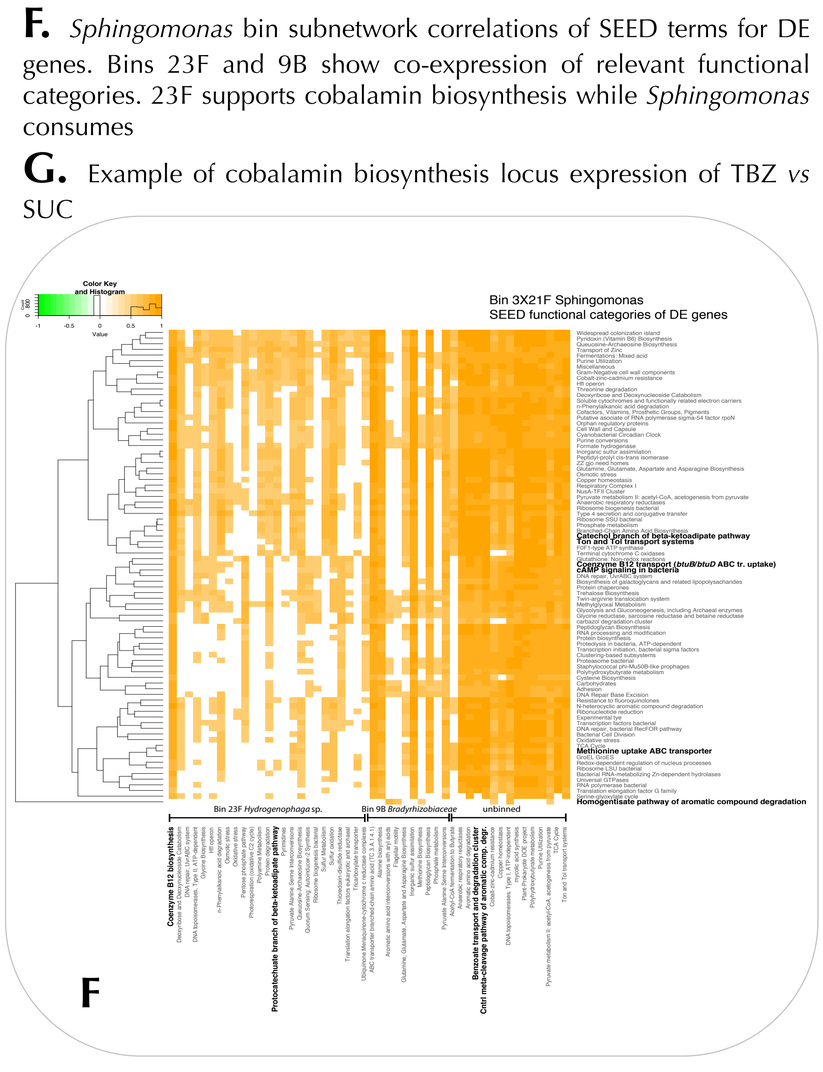
F. *Sphingomonas* bin subnetwork correlations of SEED terms for DE genes. Bins 23F and 9B show co-expression of relevant functional categories. 23F supports cobalamin biosynthesis while *Sphingomonas* consumes

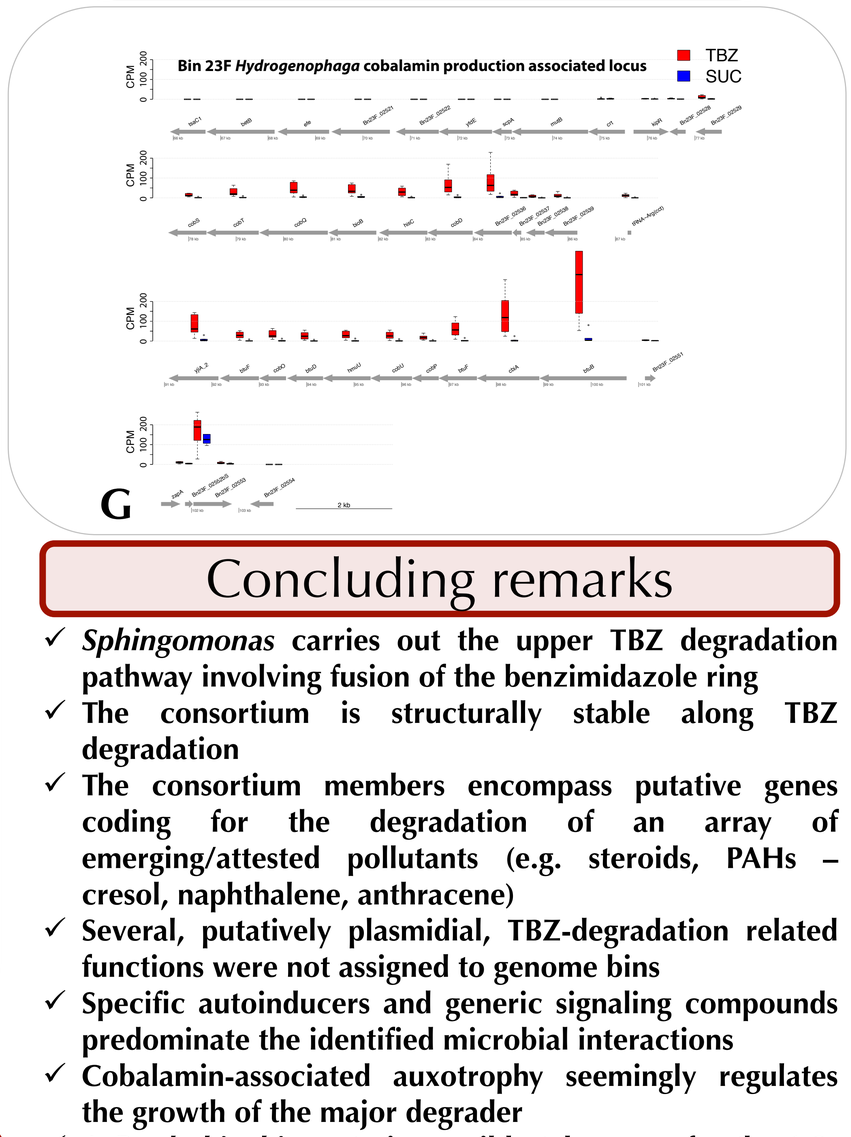
G. Example of cobalamin biosynthesis locus expression of TBZ *vs* SUC











Concluding remarks

- ✓ ***Sphingomonas*** **carries out the upper TBZ degradation pathway involving fusion of the benzimidazole ring**
- ✓ **The consortium is structurally stable along TBZ degradation**
- ✓ **The consortium members encompass putative genes coding for the degradation of an array of emerging/attested pollutants (e.g. steroids, PAHs – cresol, naphthalene, anthracene)**
- ✓ **Several, putatively plasmidial, TBZ-degradation related functions were not assigned to genome bins**
- ✓ **Specific autoinducers and generic signaling compounds predominate the identified microbial interactions**
- ✓ **Cobalamin-associated auxotrophy seemingly regulates the growth of the major degrader**
- ✓ **A *Bradyrhizobiac*e strain possibly takes over for the 1,3-thiazole-carboxamidine degradation**

17th International Symposium on Microbial Ecology, Leipzig, Germany, August 12-17, 2018