Sensitivity of pesticide biodegradation kinetics to depletion of soil microbial species richness

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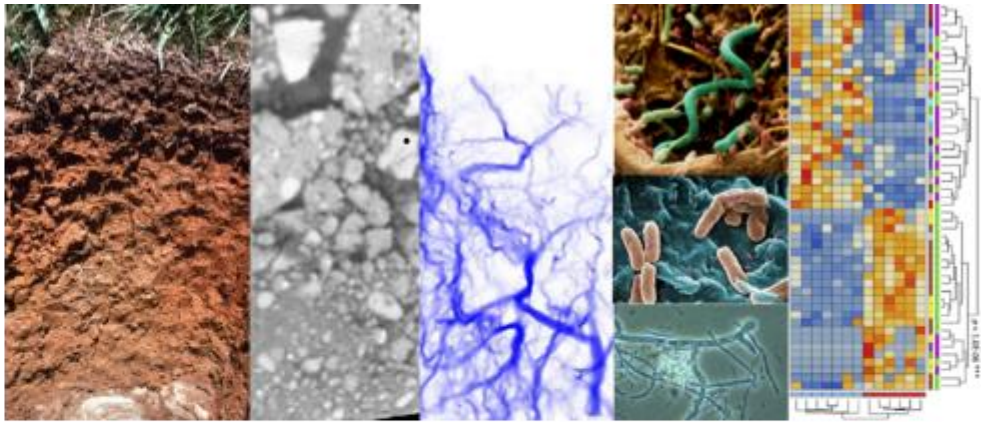
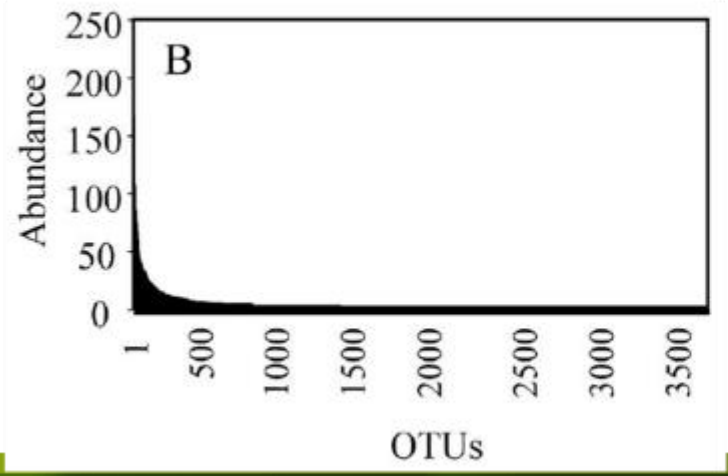
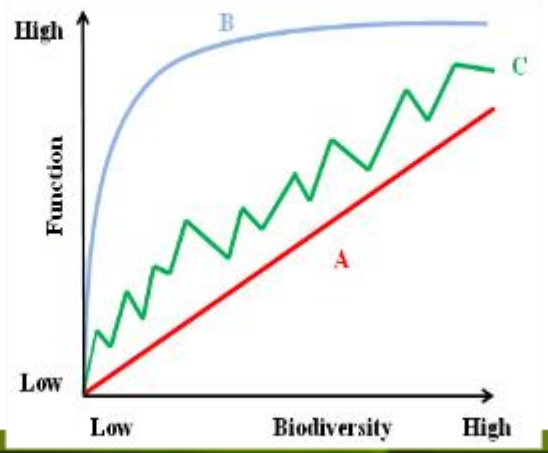
Soil Biodiversity



- The relationship between **biodiversity and ecosystem function** (BEF) has been studied for a number of decades, originally focussing on macro-organisms and above-ground communities
- BEF relationships ***in soil*** have come into focus as the extreme microbial diversity of soils became accessible (microbial fingerprinting, NGS, 'omics)
  - higher biodiversity found to confer a benefit to some functions (e.g. nitrification, denitrification, methane oxidation)
  - broad-scale functional parameters (e.g. SIR) can increase as biodiversity decreases
  - functional redundancy vs the ecological role of the rare biosphere



Elshahed *et al. Appl. Environ. Microbiol.* (2008), 74, 5422-5428.



Adapted from U . N . Nielsen *et al. European Journal of Soil Science*, (2011), **62**, 105–116

- BEF relationships

A sensitive to loss of biodiversity

B functional redundancy

C idiosyncratic response

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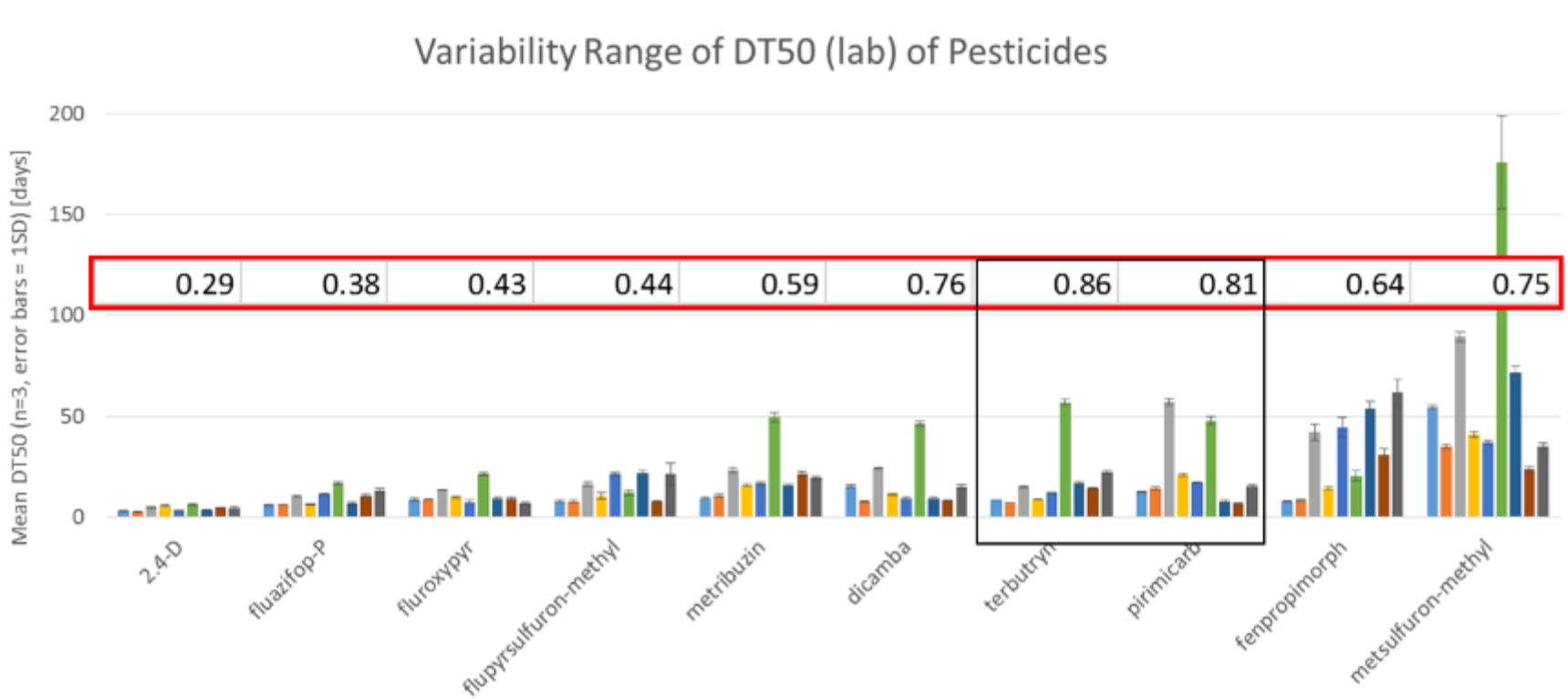
Pesticide degradation in laboratory studies

- Half-lives of degradation of 10 pesticides in 9 different soils (coloured bars, triplicate DT50s per soil)
- The coefficient of variation of the $_{mean}$DT50 across these 9 soils is shown to indicate the range within which soil half lives vary
- Much but not all of this variability can be attributed to the physical and chemical characteristics of the soil

➔ between-soil variability is compound specific and may also be determined by their BEF



Data source: Kah, Melanie, Sabine Beulke, and Colin D. Brown. "Factors influencing degradation of pesticides in soil." *Journal of agricultural and food chemistry* 55.11 (2007): 4487-4492.

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Rationale

- Test soils in degradation studies have altered microbial communities
  - soil processing affects microbial community structure and diversity
  - small soil volumes in standard lab conditions can become nutrient limited
  - this in turn affects microbial communities over time
- Lab studies may not be suitable to inform sufficiently on the true persistence range of some compounds
- New CPP development effort is ideally invested in candidates showing robust and consistent degradation (in lab and field)
- CPPs with predictable degradation in most soils allow greater precision of exposure estimates … and more robust risk assessments

Hypotheses

- Pesticide biodegradation function depends on the phylogenetic (and therefore metabolic) microbial diversity of the soil
- There may be a lack of redundancy for the degradation function of some pesticides ('specialist degraders' - high dependency - high variability of degradation)

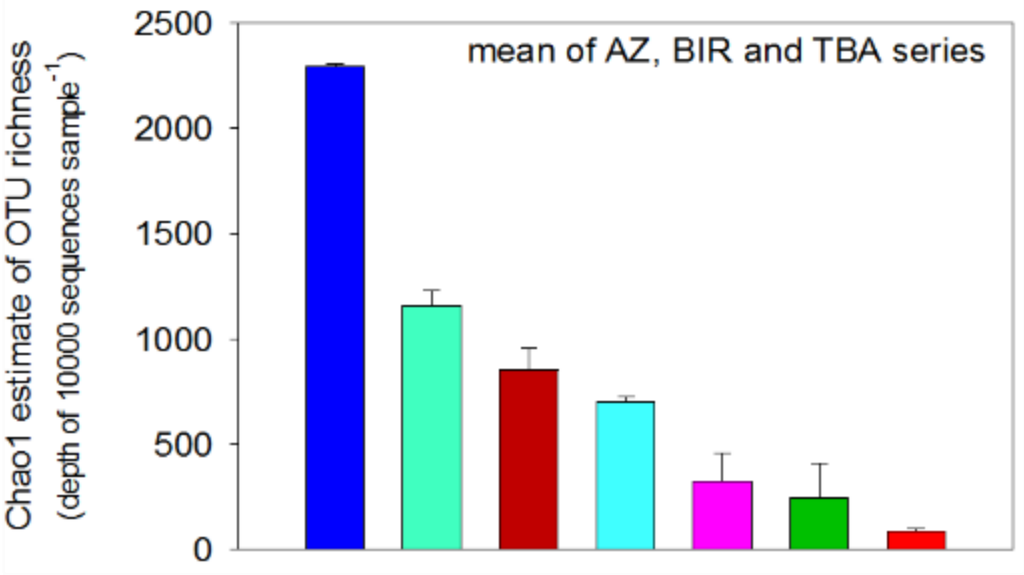
Aims

- Construct series of test soils that encompass a microbial biodiversity gradient
- Investigate if any parameters of pesticide biodegradation function are sensitive to reduction in soil microbial biodiversity

Construction of test soils of reduced biodiversity

- An initial round of experiments was conducted to optimise a dilution/inoculation methodology (adapted from Wertz et al.) for large soil volumes

- Serially diluted microbial soil suspensions of a **fresh soil**; 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, and 10^{-10}
- Inoculated into **sterilised** soil (gamma irradiation plus three rounds of autoclaving)
- 20 weeks equilibration was sufficient to allow re-colonisation and to establish equivalent levels of bioactivity (SIR, enzyme assays and qPCR)
- MySeq Amplicon sequencing confirmed a successive reduction of microbial diversity was achieved



	Fresh	10^{-2}	10^{-4}	10^{-6}	10^{-8}	10^{-10}	γ-irr
% species lost	0	45	54	66	83	88	97

Wertz, S., Degrange, V., Prosser, J. I., Poly, F., Commeaux, C., Freitag, T., Guillaumaud, N. & Roux, X. L. (2006). Maintenance of soil functioning following erosion of microbial diversity. *Environ Microbiol*, **8**, 2162-9.

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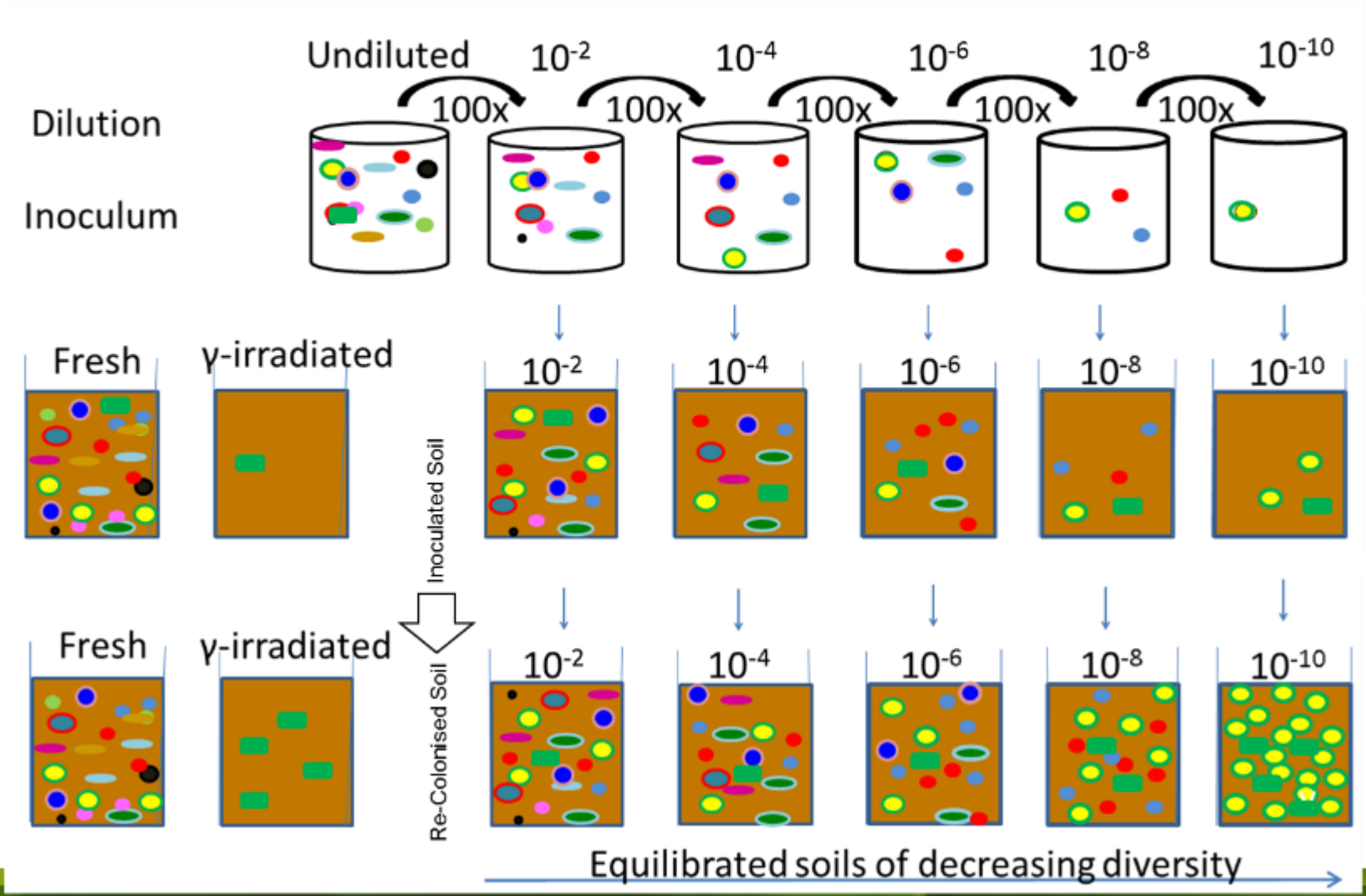
Construction of a microbial diversity gradient



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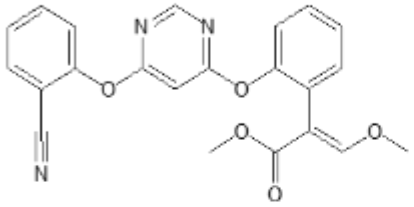
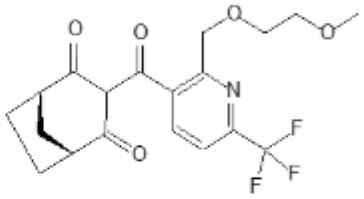
Degradation studies using soils of decreasing microbial diversity

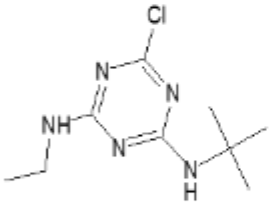
Azoxystrobin



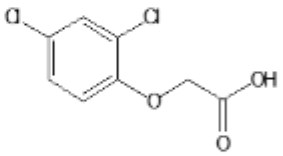
Bicyclopyrone

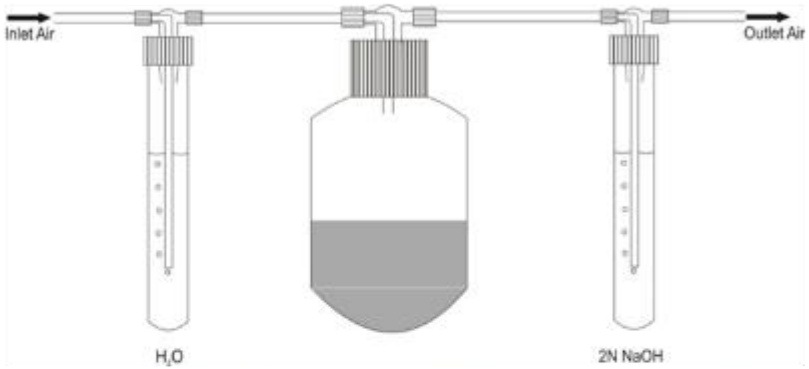


Terbuthylazine



2,4-D

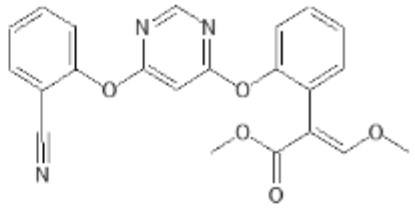


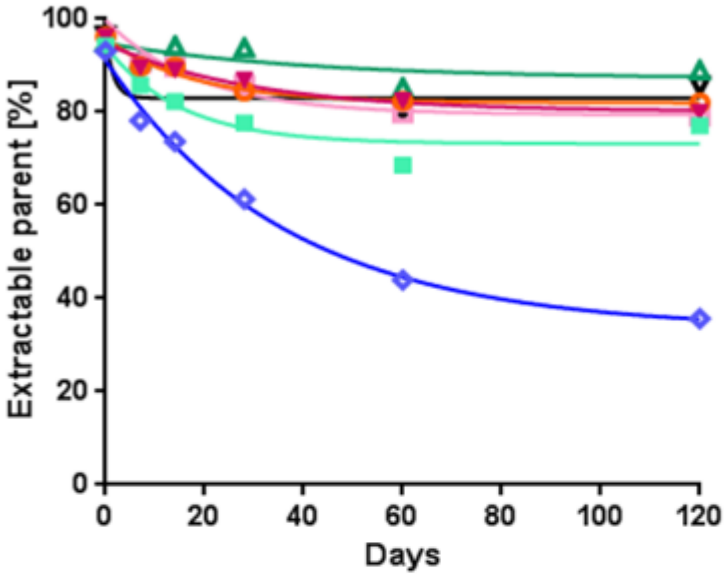


- Soils are solvent extracted and extracts analysed by
  - LSC to quantify extractable ^{14}C residue (parent + transformation products)
  - HPLC with radiodetection to quantify % of parent remaining
- Analysis of trapped $^{14}CO_2$ and post-extraction solids = non-extractable residue
- Obtain a full mass balance

OECD Test No. 307: Aerobic and Anaerobic Transformation in Soil
DOI:http://dx.doi.org/10.1787/9789264070509-en

Azoxystrobin





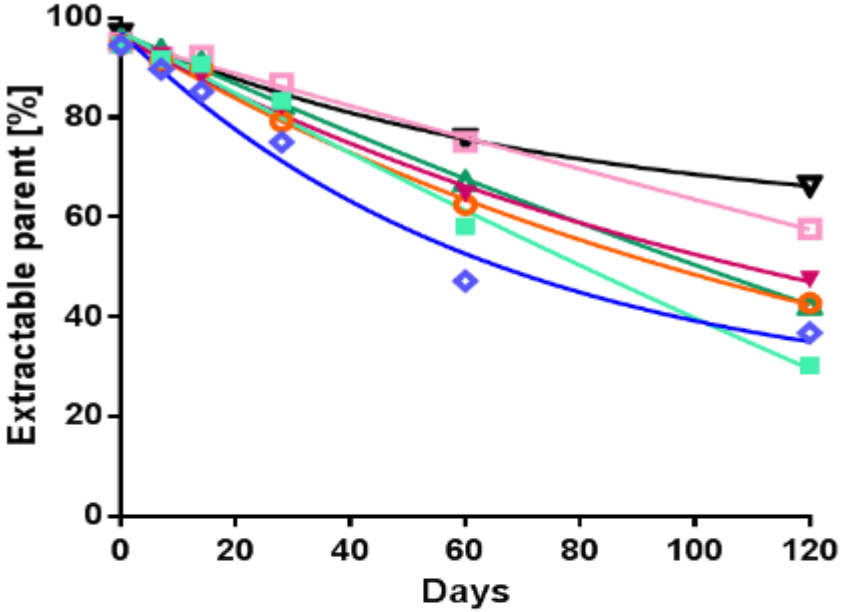
	k	DT_{50} [days]
Fresh	0.009	77
10^{-2}	0.001	464
10^{-4}	0.001	542
10^{-6}	0.001	649
10^{-8}	0.001	417
10^{-10}	0.001	1191
γ-irradiated	<0.001	> 10 years

- The reported range of $DT50_{lab}$ of 35.2-248 days *
- In our study, the DT50 in fresh soil was 77days
- Soil photolysis contributes to AZ degradation in the field - some of the $DT_{50\ field}$ range can be attributed to the influence of light
- For field trials with incorporation a $DT50_{field}$ range of 121-262 days remains

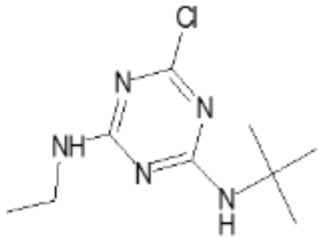
- All parameters of AZ degradation function (DT50, k and %parent remaining) show some impairment compared to the fresh control for all dilutions
- This indicates high dependency on biodiversity

* *European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance azoxystrobin. EFSA Journal 2010;8(4):15421542. [110 pp.]. doi:10.2903/j.efsa.2010.1542.*

Terbuthylazine



	k	DT_{50} [days]
Fresh	0.01	75
10^{-2}	0.01	77
10^{-4}	0.01	115
10^{-6}	0.01	101
10^{-8}	0.001	166
10^{-10}	0.01	104
γ-irradiated	<0.001	> 10 years



- DT50 ranges in lab ($DT50_{lab}$ 65 - 167 days) and field ($DT_{50\ field}$ 10 - 148 days)* overlap well
- In our study, the DT50 in fresh soil was 75 days

- In the 10^{-2} soil, which retained less than half of the estimated OTU richness of the fresh soil, TBZ degradation was preserved according to all three parameters of degradation function (DT50, k and %parent remaining)
- The 10^{-10} soil degraded nearly 20% more parent than the 10^{-6} and 10^{-8} …dependency on community composition?

European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance terbuthylazine. EFSA Journal 2011;9(1):1969. [133 pp.]. doi:10.2903/j.efsa.2011.1969

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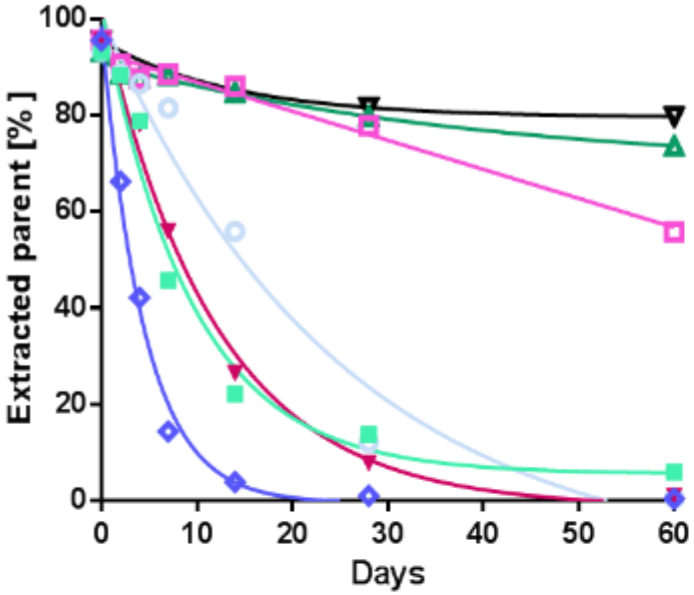
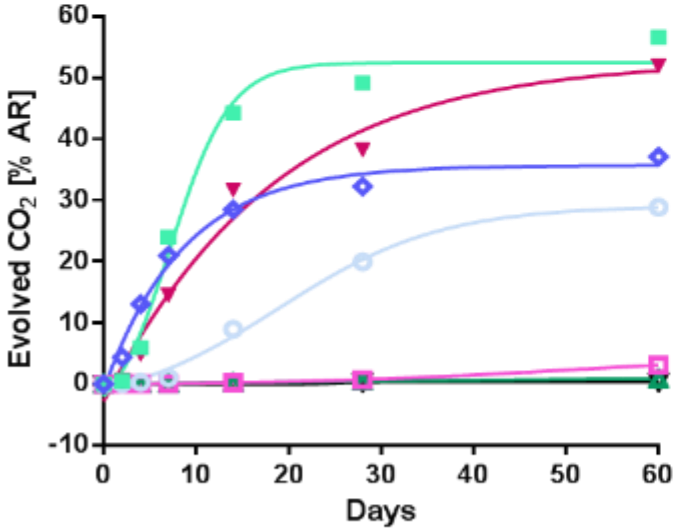
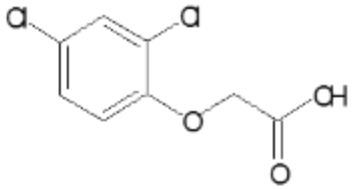
2,4-D kinetics





	k	DT_{50} [days]
Fresh	0.227	3
10^{-2}	0.092	7
10^{-4}	0.085	8
10^{-6}	0.052	13
10^{-8}	0.008	87
10^{-10}	0.004	188
γ-irradiated	0.003	238



- The reported lab DT50 range ($DT50_{lab}$ 1.2 – 94.6 days) is much wider than that in the field ($DT_{50\ field}$ 22 - 38 days) *
- In our study, the DT50 in fresh soil was 3 days

- Degradation function was maintained down to the 10^{-6} soil (according to DT50, k, %parent remaining AND formation of $^{14}CO_2$)
- Mineralisation of 2,4-D was more comprehensive in the 10^{-2} and 10^{-4} soils compared to the fresh

EFSA (European Food Safety Authority), 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance 2,4-D. EFSA Journal 2014;12(9):3812, 78 pp. doi:10.2903/j.efsa.2014.3812

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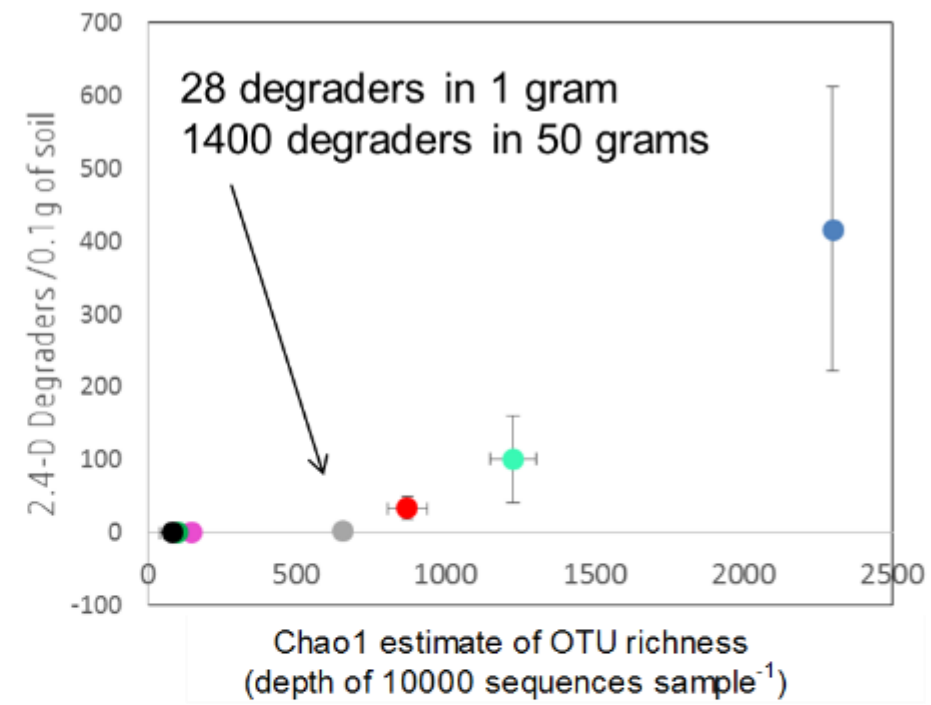
Abundance of 2,4-D degraders by MPN

- Most Probable Number (MPN) experiment
- Cell-based assay, estimates the number of degraders present in a soil
- A reduction in 2,4-D degraders, coherent with the overall diversity erosion, was observed
- Degrader numbers were below the level of detection in the 10^{-8}, 10^{-10} and gamma irradiated soils
- The 10^{-6} soil contained an estimated ~1400 degraders … and still achieved near complete removal of extractable 2,4-D



Cochran, W. G. (1950). Estimation of bacterial densities by means of the "most probable number".Biometrics, 6, 105-16.
Alexander, Martin. "Most probable number method for microbial populations."
Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties methodsofsoilan2 (1982): 815-820

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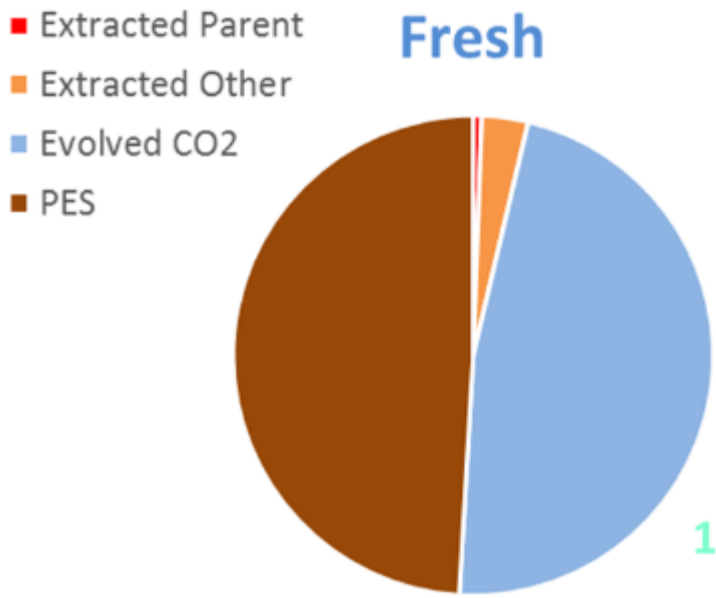
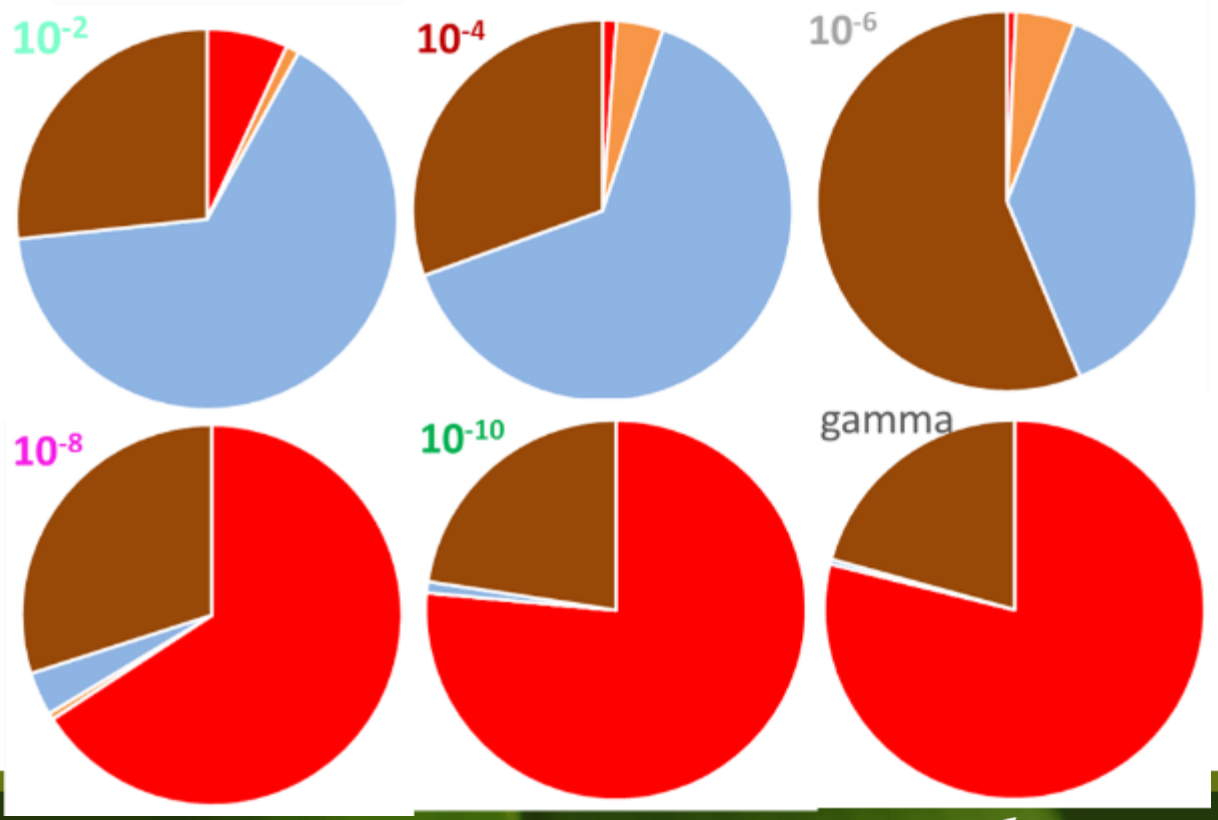
Biodiversity depletion affects distribution of 2,4-D residues





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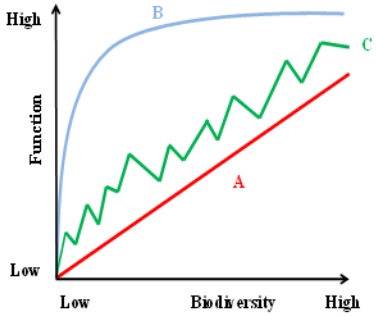
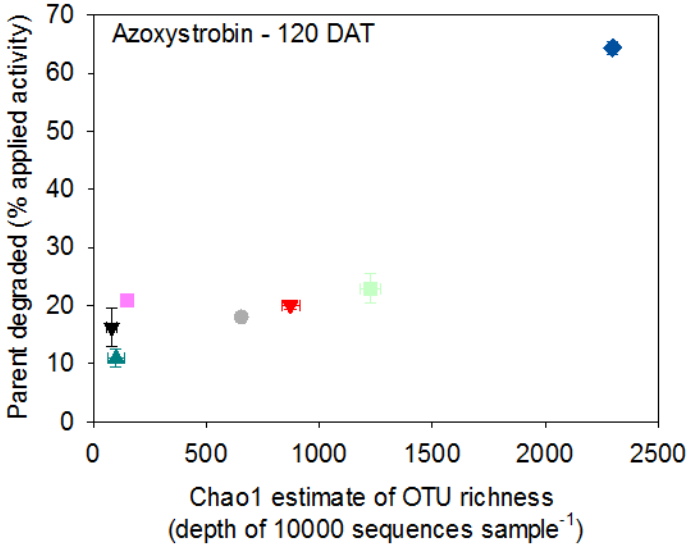
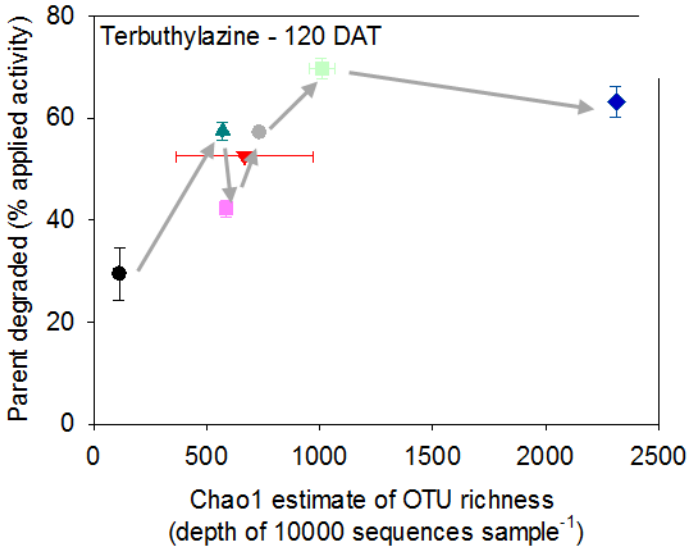
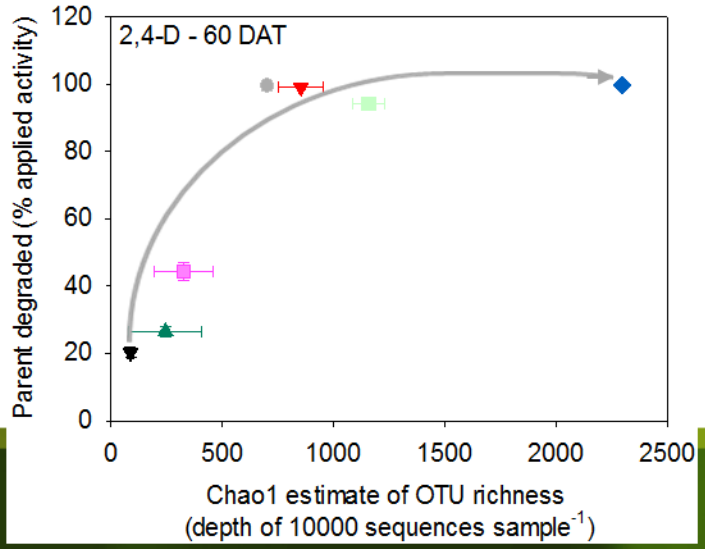
What B-EF relationships did we see?

(%parent degraded as parameter of degradation function)









Fresh
10^{-2}
10^{-4}
10^{-6}
10^{-8}
10^{-10}
Gamma

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Conclusions

- Hypothetical biodiversity-function relationship types postulated for other systems exist also for hyper-complex soil communities
- The degree of sensitivity to microbial depletion is compound specific
- Functional redundancy for the degradation of some pesticides may be limited
- The approach used here may offer utility in CPP R&D;
  - identify and focus on candidate compounds where degradation function is resistant to microbial depletion

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University of Reading

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