Size matters: the effect of scale in chemical biodegradation studies

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Introduction

- Environmental persistence of pesticides is significant concern
- OECD tests for aquatic biodegradation lack environmental realism.
- The degradation of chemicals in the simulation tests tends to be slower than in field trials, and there is more variation and inconsistency.
- The problems with tests are likely to be caused by difficulty of simulating real environment in a small microcosm, due to a range of issues.
- This dominant problem is something that can be called the “Effect of Scale” – a difficulty of reducing real environment down to a small system.
- OECD Test 308 – a simulation test for chemical transformation in aquatic systems is the focus of this study.

Aims

- To determine how the scale of test system affects the rate of biodegradation.
- To test if it differs when chemicals with contrasting properties are used.
- To investigate how and why difference occurs using biogeochemical and microbial community analyses.

Experimental setup

- Vessels of 100x volume difference were used
- Five experiments with different pesticides, chosen based on contrasting properties. Radiolabelled chemicals were used in microcosms and non-labelled in flumes
- River water and sediment was collected from the river Dene in Wellesbourne, UK

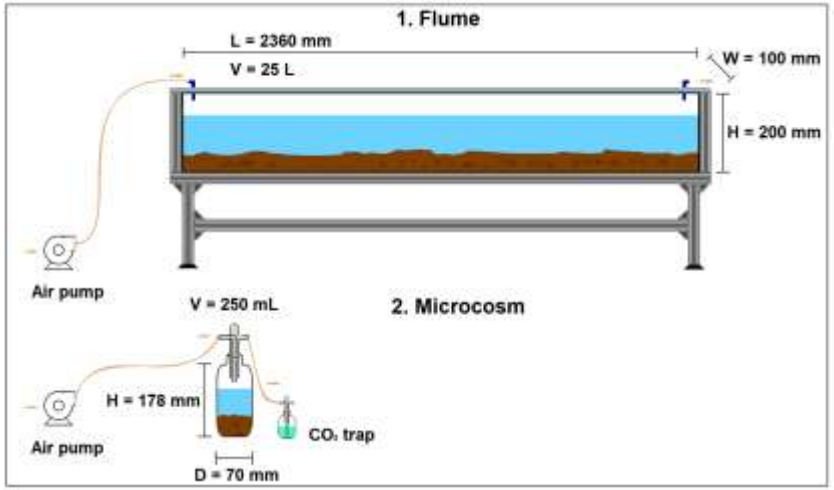


Fig. 2. A diagram showing experimental setup of flumes and microcosms.

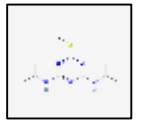
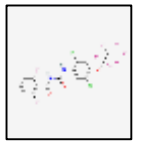
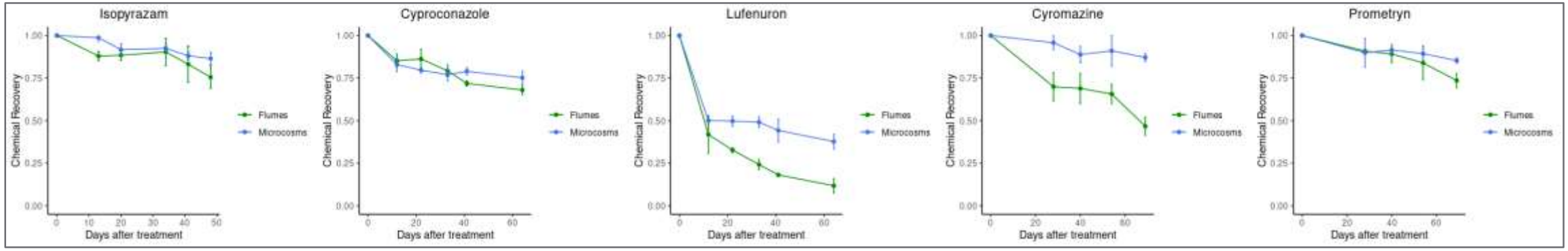
Chemical	Structure	Description	Degradation kinetics	Mechanism of Action
Isopyrazam		Isopyrazam ($C_{20}H_{23}F_2N_3O$) is a carboxamide fungicide. Molecular weight – 359.4 g/mol	DT_{50} – 628 days Log K_{ow} – 4.25	Inhibition of succinate dehydrogenase, disrupting cellular respiration in fungal mitochondria.
Prometryn		Prometryn ($C_{10}H_{19}N_5S$) is a diamino-1,3,5-triazine herbicide. Molecular weight: 241.36 g/mol	DT_{50} >100 days Log K_{ow} – 3.51	Prometryn selectively inhibits photosystem II in plants, particularly in broadleaves and grasses.
Cyromazine		Cyromazine ($C_6H_{10}N_6$) is a triazine insecticide. Molecular weight: 166.18 g/mol	DT_{50} – 143 days Log K_{ow} – 0.069	Cyromazine is an insect growth regulator, affecting nervous system in larvae of certain insects.
Lufenuron		Lufenuron ($C_{17}H_8Cl_2F_8N_2O_3$) is a chlorinated benzoylurea insecticide. Molecular weight: 511.1 g/mol	DT_{50} = 112 days. Log K_{ow} 5.12	Lufenuron inhibits production of chitin in certain species of insects.
Cyproconazole		Cyproconazole ($C_{15}H_{18}ClN_3O$) is an azole fungicide. Molecular weight: 291.77 g/mol	DT_{50} >365 days. Log K_{ow} 3.01	Fungal cell wall synthesis inhibitor.

Table 1. A summary of chemical characteristics of pesticides that were used in this study. Diagrams and chemical data from Pesticide Properties DataBase (PPDB) of University of Hertfordshire (http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm, accessed on 16/18/2021.

Analytical methods

- Chemicals were dissolved in solvents and added to test systems.
- Samples were taken at regular intervals over periods of ~ 60 days.
- Total recovery was measured by LC-MS and Radiochromatography after solvent extraction.
- Biogeochemical measurements were done using probes and spectrophotometric methods.
- Biomass was measured by Flow Cytometry.
- 16S rRNA Sequencing for microbial community analysis

Results – chemical analysis



$p = 0.047$ $p = 0.002$ $p < 0.001$ $p = 0.021$ $p = 0.453$

Fig. 3. Total recovery of chemicals in the experiments with Isopyrazam, Cyproconazole, Lufenuron, Cyromazine and Prometryn.

- Flume degradation rates were faster and significantly different from microcosms for all chemicals except for Prometryn, based on repeated measures ANOVA.
- The estimates of chemical half life DT50 (the time it takes for 50% of chemical to degrade) are much shorter in flumes than microcosms for all 5 chemicals. A Single First-Order kinetics model was used for this estimate.

Estimated DT50 (days)					
	Isopyrazam	Cyproconazole	Lufenuron	Cyromazine	Prometryn
Flumes	157	113	14.4	72	174
Microcosms	228	168	39.3	345	328

Table 2. An estimate of time (in days) it will take for 50% of chemical to decay, based on Single First-Order kinetics model.

Results – metadata

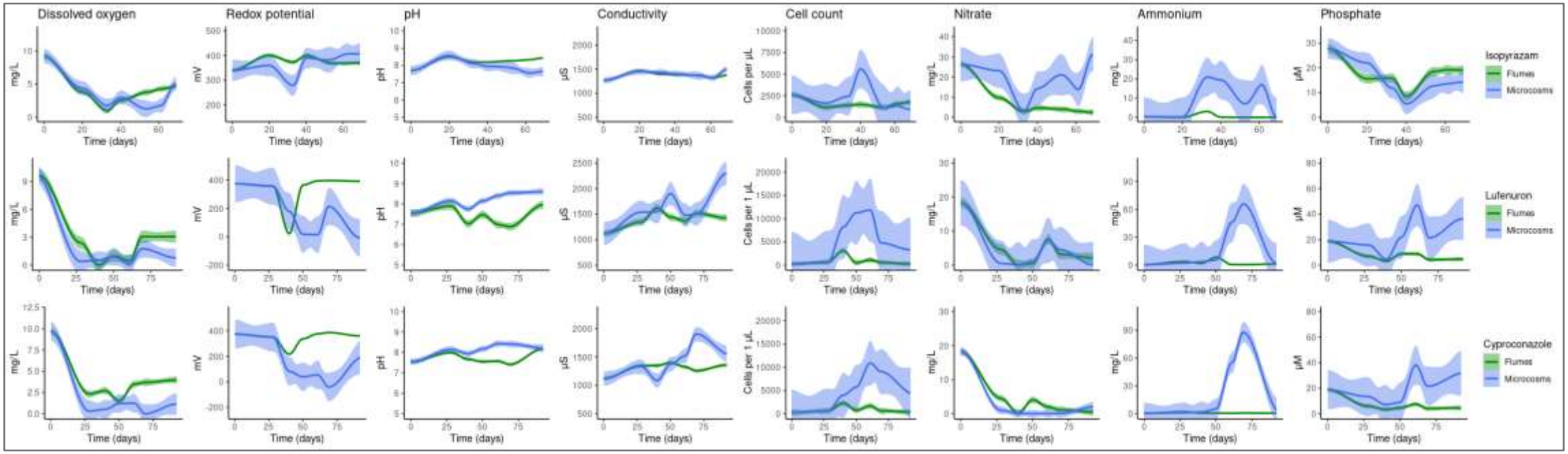


Fig. 4. Isopyrazam, Lufenuron and Cyproconazole experiment metadata, displayed with LOESS Scatterplot estimation based on local regression, shaded areas are 95% confidence intervals.

- Flume data is much less variable than microcosm data
- The extent of total changes over time is considerably smaller in flumes
- Cell count and ammonium are consistently higher in microcosms, whereas redox potential and oxygen levels tends to be higher in flumes

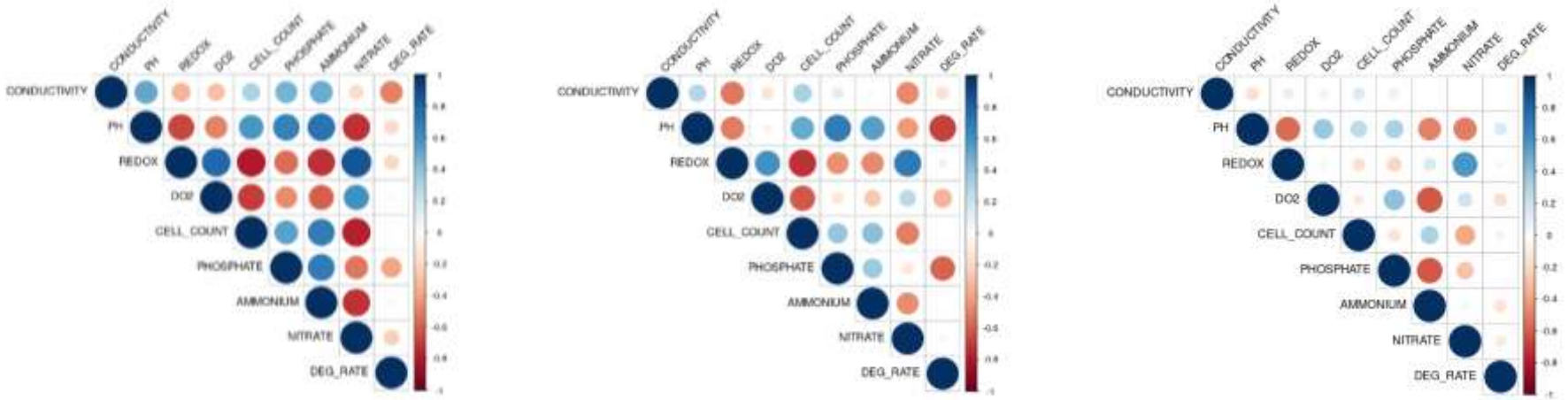
Summary of variation

Average coefficient of variation (%)								
Chemical	**Type**	**pH**	**Oxygen**	**Redox**	**Conductivity**	**Phosphate**	**Ammonium**	**Nitrate**
Lufenuron	Microcosms	2.12	130.64	145.27	16.97	63.13	50.97	107.65
Lufenuron	Flumes	1.84	20.60	17.37	1.11	21.98	23.76	40.56
Cyproconazole	Microcosms	0.79	101.30	123.21	18.57	67.03	38.50	68.18
Cyproconazole	Flumes	1.10	13.83	2.47	2.29	27.26	32.24	33.14
Isopyrazam	Microcosms	2.67	46.01	8.91	5.59	25.87	78.61	25.87
Isopyrazam	Flumes	0.47	15.90	2.06	1.91	11.03	76.34	11.03

Table 3. Coefficient of variation of a range of measured parameters in experiments with Lufenuron, Cyproconazole and Isopyrazam. Higher values in each experiment are in red and lower respective value in green.

- Coefficient of variation (a statistical measure of data dispersion) for most of the measurements is higher in microcosms than in the flumes.

Correlations between measurements



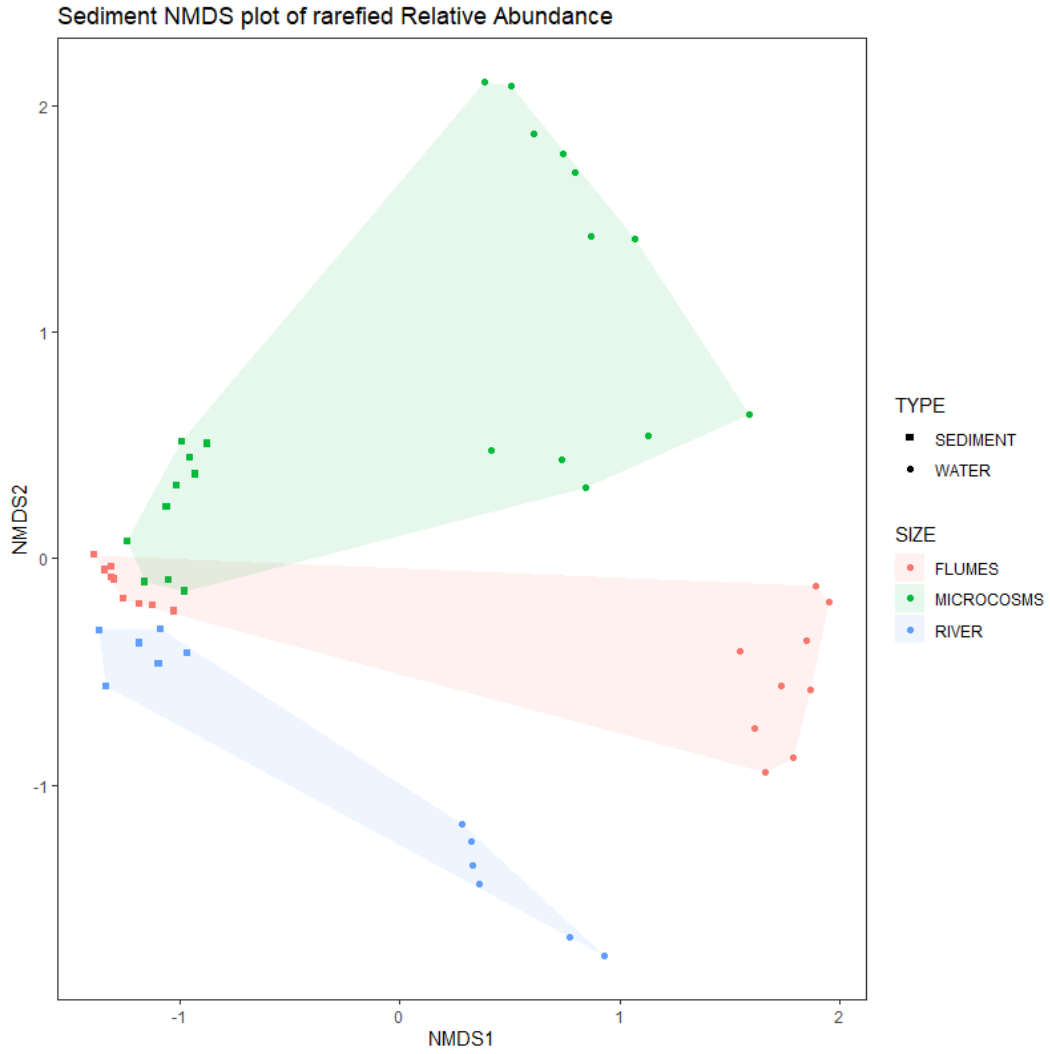
Cyproconazole **Lufenuron** **Isopyrazam**

Fig. 5. Correlation heatmaps of nutrient data, probe measurements and degradation rates in experiments with Cyproconazole (A), Lufenuron (B) and Isopyrazam (C).

- Correlation matrices show the relationship between environmental factors.
- Ammonium and Nitrate dominated ecosystem types are opposite in many ways.
- Degradation rate correlations differ between different chemicals, but it tends to be negatively correlated with Phosphate which is associated with ammonium-rich systems.

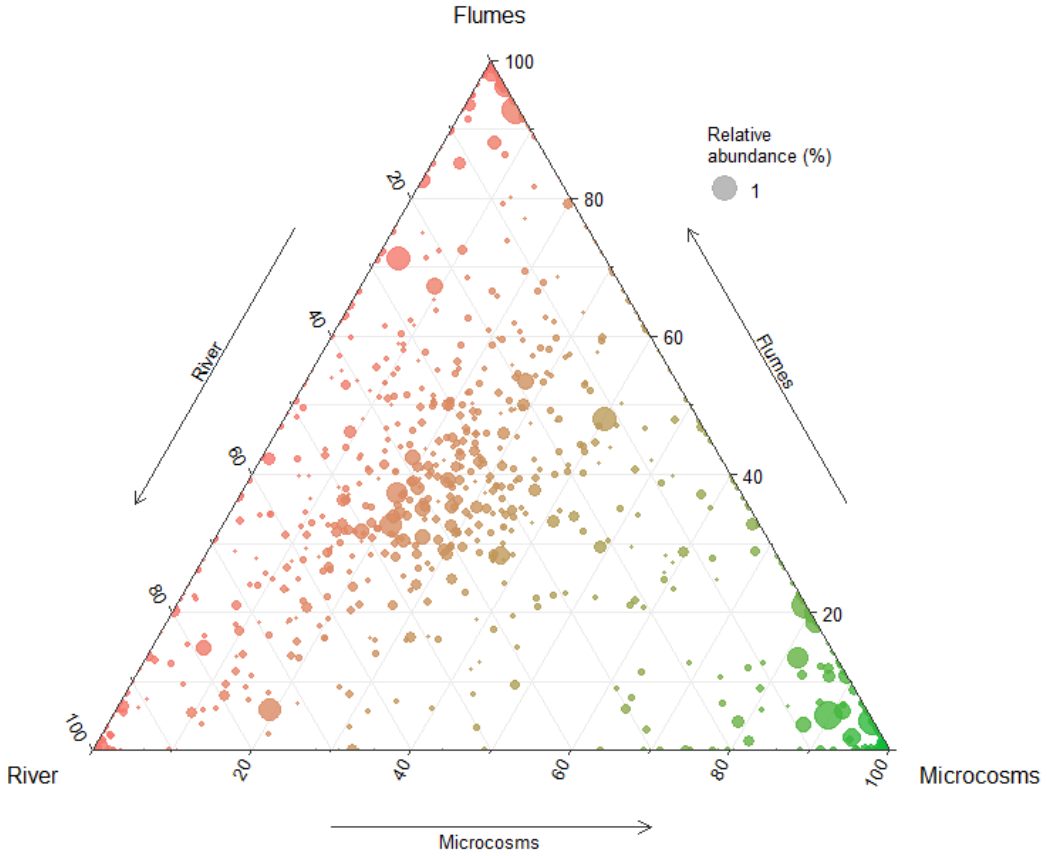
Microbial diversity

- Total unique Amplicon Sequence Variants identified: **27098**
- Non-metric Multidimensional Scaling Plot (NMDS) shows the composition of microbial community based on Relative Abundance of microbial species (Beta Diversity)
- Community composition in flumes is closer to River, both in sediment and water than Microcosms
- Microcosms show highest variation in microbial composition, both in sediment and water
- Microbial community in water phase is different to sediment in all cases

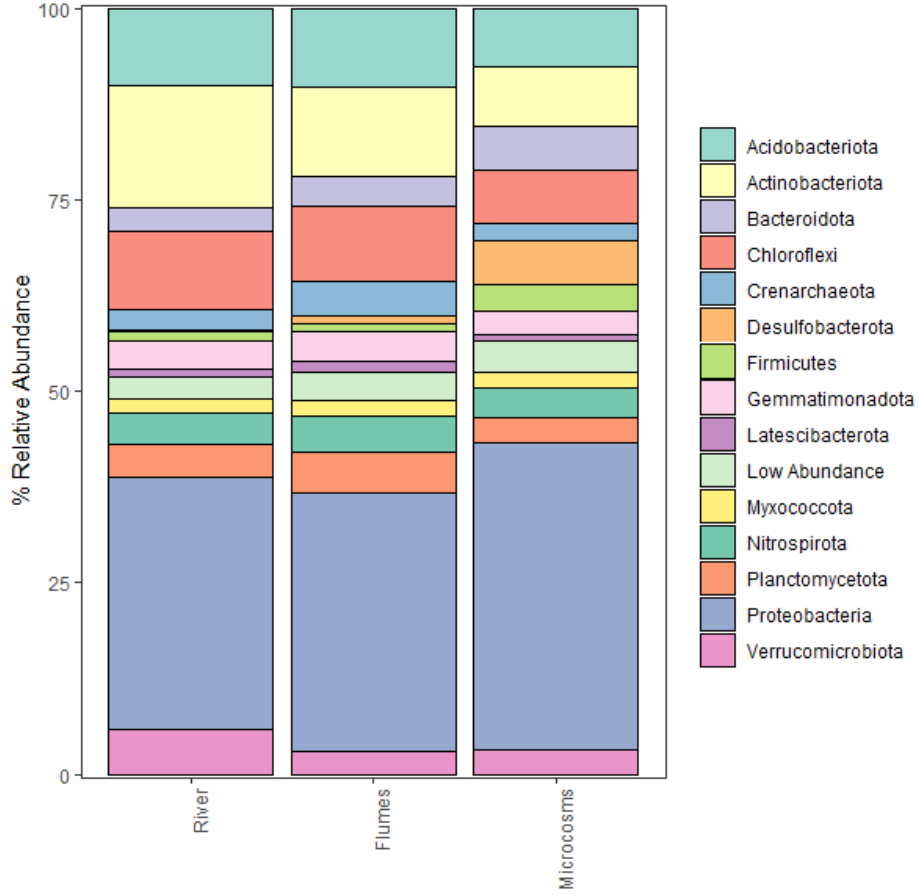


Microbial community

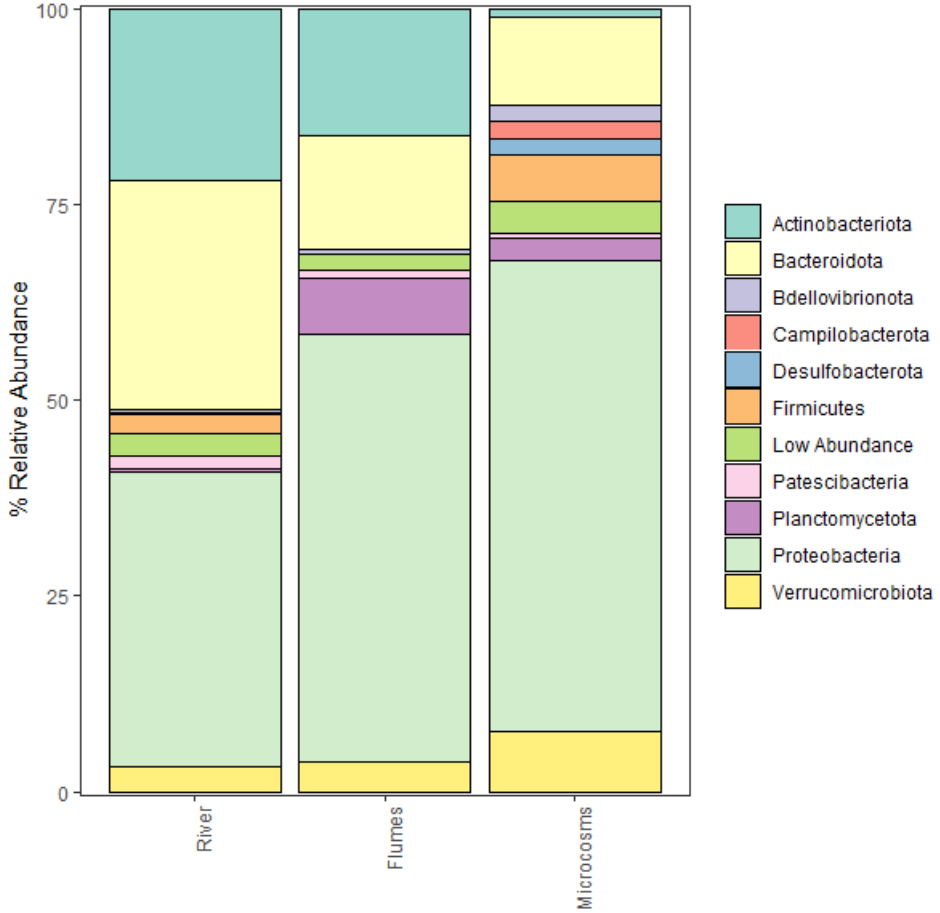
- Ternary plot shows relationship between 3 sources – River, Flumes and Microcosms, with sediment and water data combined
- A larger part of microbial community tends to be present in both Flumes and River but not in Microcosms
- While both Flumes and Microcosms tend to develop some system-specific microbial species in high abundance, the community in Microcosms seems to be more distinct and isolated



Microbial community composition



Sediment



Water

Conclusion

- Size matters in chemical degradation tests!
- Chemical biodegradation in larger systems is consistently faster than in smaller microcosms
- Variability of all measured parameters is greater in smaller systems, with larger overall changes, therefore larger systems are more stable and consistent
- Small systems differ significantly from real environment with ammonium-rich, high cell count, lower oxygen and redox potential conditions
- Microbial community of larger test system is more consistent and is much closer related to the real environment than in small microcosms
  - Microcosms are more prone to develop a distinct microbial community
  - There is less consistency in the community composition of microcosms than in flumes

End

Questions?

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