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

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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_

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, which 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

<table>
<thead>
<tr>
<th>% species lost</th>
<th>Fresh</th>
<th>$10^{-2}$</th>
<th>$10^{-4}$</th>
<th>$10^{-6}$</th>
<th>$10^{-8}$</th>
<th>$10^{-10}$</th>
<th>$\gamma$-irr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>45</td>
<td>54</td>
<td>66</td>
<td>83</td>
<td>88</td>
<td>97</td>
</tr>
</tbody>
</table>

Construction of a microbial diversity gradient

Equilibrated soils of decreasing diversity
Degradation studies using soils of decreasing microbial diversity

- 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
Azoxystrobin

The reported range of DT50_{lab} of 35.2-248 days *

In our study, the DT50 in fresh soil was 77 days

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

● DT50 ranges in lab (DT50[lab] 65 - 167 days) and field (DT50[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?
2,4-D kinetics

<table>
<thead>
<tr>
<th></th>
<th>k</th>
<th>DT$_{50}$ [days]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0.227</td>
<td>3</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>0.092</td>
<td>7</td>
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<tr>
<td>$10^{-4}$</td>
<td>0.085</td>
<td>8</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>0.052</td>
<td>13</td>
</tr>
<tr>
<td>$10^{-8}$</td>
<td>0.008</td>
<td>87</td>
</tr>
<tr>
<td>$10^{-10}$</td>
<td>0.004</td>
<td>188</td>
</tr>
<tr>
<td>$\gamma$-irradiated</td>
<td>0.003</td>
<td>238</td>
</tr>
</tbody>
</table>

- The reported lab DT50 range (DT$_{50\text{lab}}$ 1.2 – 94.6 days) is much wider than that in the field (DT$_{50\text{field}}$ 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

*Note: The asterisk symbol (*) typically denotes a note or reference, but in this context, it appears to be a part of the text. It might be a misprint or a special notation within the document.
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

Biodiversity depletion affects distribution of 2,4-D residues
What B-EF relationships did we see? (%parent degraded as parameter of degradation function)
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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