# Supporting Information

## Biodegradation of volatile chemicals in soil – Separating volatilization and degradation in improved test setup (OECD 307)

Prasit Shrestha[1,2], Boris Meisterjahn[1*], Michael Klein[1], Philipp Mayer[2], Heidi Birch[2], Christopher B. Hughes.[3], Dieter Hennecke.[1]

[1]Fraunhofer IME-AE, Auf dem Aberg 1, 57392 Schmallenberg Germany.

[2]Department of Environmental Engineering, Technical University Denmark, 2800 Kongens. Lyngby, Denmark

[3]Ricardo Energy and Environment, Harwell, OX11 0QR, United Kingdom

## Table of Contents



## List of Figures

## List of Tables

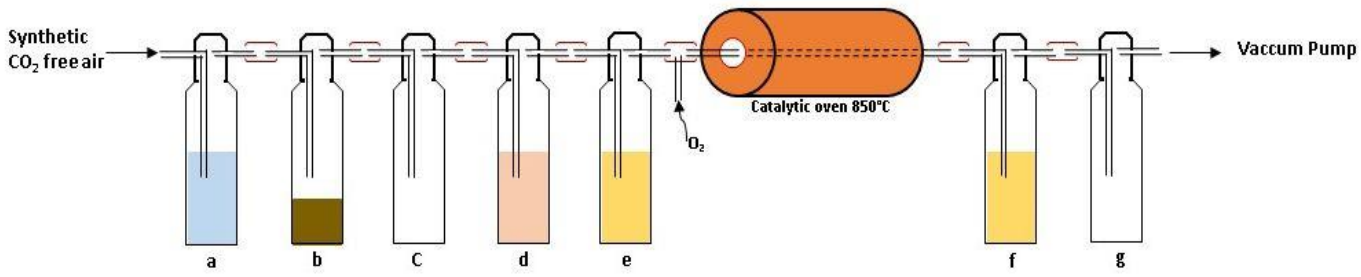
## S1) Measured parameters of soils used during this project

*Table S1 Soil texture and additional parameters of the soil used during this project.*

| Soil | Sand % | Silt % | Clay % | Soil type | Org. C % | pH ($CaCl_2$) | WHC g/kg | $CEC_{eff}$ (mmol/kg) |
|---|---|---|---|---|---|---|---|---|
| **01-A** | 76.70 | 17.20 | 6.10 | loamy sand | 0.73 | 5.73 | 291.0 | 17.90 |
| **02-A** | 2.30 | 82.00 | 15.70 | silty loam | 1.01 | 6.69 | 471.0 | 53.40 |
| **03-G** | 17.71 | 57.49 | 24.80 | silty loam | 2.80 | 6.23 | 734.0 | 73.50 |
| **04-A** | 82.70 | 12.70 | 4.60 | loamy sand | 2.48 | 5.96 | 382.0 | 37.30 |

WHC: Water Holding Capacity

## S2) Schematic diagram of flow-through setup connected with a catalytic oven




a: deionised water
b: Soil applied with test substance
c: empty bottle (safety trap)
d: ethyleneglycol trap
e: sodium hydroxide trap (before oven)
f: sodium hydroxide trap (after oven)
g: empty bottle (safety trap)

*Figure S1 Schematic diagram representing a modified flow-through setup used during this project. A constant stream of water saturated synthetic air was passed over the soil samples to keep the soil aerobic and the outgoing gas was bubbled through a series of adsorption traps and additionally through a tube furnace (850°C, copper oxide as catalyst in the tubes) to capture the possibly formed $^{14}CO_2$ in a traps behind the oven.*

## S3) A picture of test setup 1

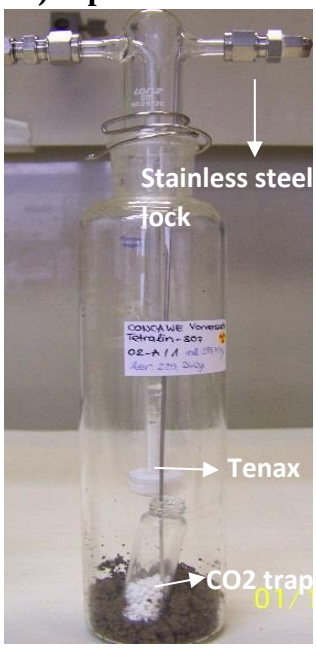



*Figure S2 A Picture of test setup 1 used to test $^{14}C$ labelled tetralin during this study.*

## S4) Mass Balance of $^{14}C$ labelled tetralin in three different test setups.

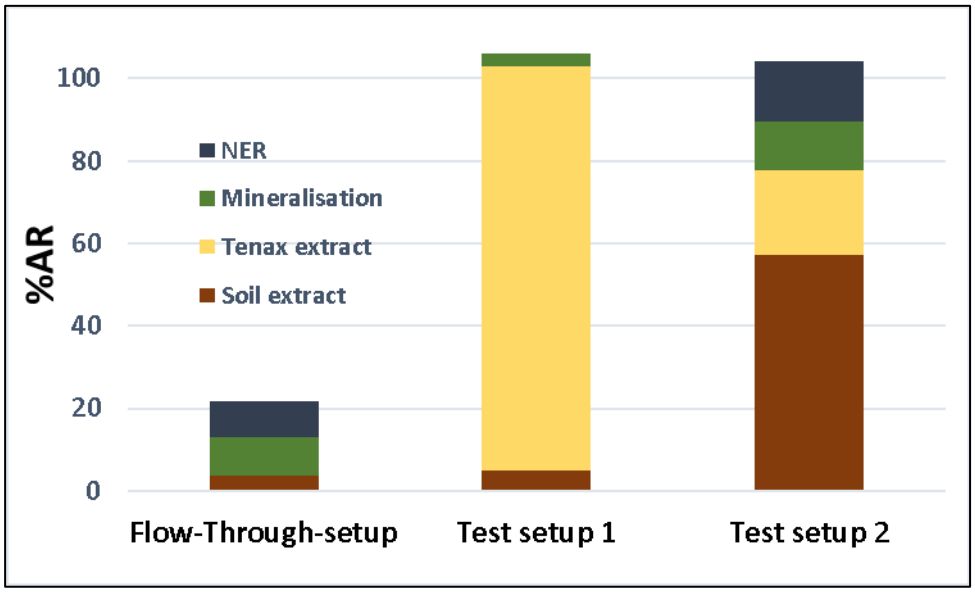



*Figure S3 Different pools of radioactivity observed in the degradation study carried out with $^{14}C$ labelled tetralin in three different test setups. The results after 14 days incubation with 02-A soil illustrate the difference in mass balance obtained between different test setups.*

**S5) Sterile samples**

Sterile samples were prepared by autoclaving 50 g (dW) soil at 121°C for 20 min for 2 cycles (Dx-65 Systec) and applied with $^{14}C$ labelled test chemical. The sterile samples were also prepared as per test setup 2 but without NaOH trap and incubated at similar test conditions as the other test flasks. The incubation time for the sterile samples were similar to that of non-sterile samples but only 3 samples in duplicates were scheduled between the incubation periods (start, middle and end of the study).

**S6) Microbial biomass measurements**

Soil samples in duplicates applied with and without co-solvent were prepared according to test setup 2 but without tenax and $CO_2$ absorption trap. These soil samples were used to see the effect of solvent and incubation conditions on the microbial biomass during the test and were sampled at the start, middle and end of the study. These samples were also oxygenated, if the oxygen saturation measured in the reference samples were lower than 15%. The microbial biomass measurement were based on the substrate induced respiration (SIR) method and was performed according to standard DIN ISO 17155.

**S7) Oxygen measurement in the headspace of the closed setup (test setup 2)**

**Fixation of Oxygen sensor spots and lens adaptor**

Firstly, a contactless oxygen sensor spot ($OXSP_5$, Pyroscience) was attached to a 100mL sample bottle (approximately 12cm from the base of the bottle), to its inner wall using a silicone based glue. After the glue was completely dried, a lens spot adapter (SPADLNS, Pyroscience) was positioned exactly over the sensor spot on the outer wall of the sample bottle and fixed firmly using its belt.

**Calibration of the sensor spots**

An optical fibre (SPFIB, Pyrocience) was used to connect the adaptor with the optical oxygen meter (FireSting O2), which was also connected to an external temperature sensor. The oxygen meter was then connected to the laptop using a USB cable and operated with software (Pyro Oxygen logger). Each sensor spot was assigned a special sensor code, which was entered in the

software before its calibration. The sensor spots were calibrated using a 2-point calibration at 0% $O_2$ saturation and ambient air by filling in the bottles with nitrogen and air, respectively. The calibration file generated by the software for each of these sensors was then stored in the laptop.

**Preparation of the reference sample**

After the calibration, 50g of dry soil was added in each of the bottles without contaminating the sensor spots. One set of the soil sample was applied with solvent (same amount used for applying $^{14}C$ test chemical in test samples) and the other without solvent. The bottles were then closed with the same insert head as in test setup 2 but without a NaOH flask attached. The reference samples were incubated in similar condition as the other test samples (20$^{o}$C at dark)

**Oxygen measurement**

For the oxygen measurements in the headspace of the reference samples, the lens adaptor was connected to oxygen meter as described above. The calibration file associated with the sensor was assigned to the software and the oxygen measurement was performed for 3 min until a stable signal was reached.

**S8) Headspace air stripping during the sampling date**

At sampling date, the sample bottle was removed from the incubation room and the tenax end of the sample bottle was connected to a pump (Air check Sampler, Model 224-PCXR7) and a gas meter (Ester Handel, GmBH) using a pipe. For 20 sec the headspace air was stripped out without opening the other end of the sample bottle. For the remaining 4 min 40 sec, the air was stripped out by loosening the swagelok screws on the other end. On average 0.76±0.06 L (N=74, Decane main test) of air was stripped out during this procedure from each sample in a total time of 5 min. After this step, the sample bottle was opened and the NaOH bottle and the soil were taken for further processing steps.

## S9) Extraction of the soil

*Table S2 List of soil extraction methods applied during this project*

| **Test chemical** | **Number of extraction/ Shaking time** | | |
|---|---|---|---|
| **Tetralin** | 1st / 30min | 2nd /30min | 3rd /18hrs |
| **Decane** | 1st / 30min | 2nd /30min | 3rd /18hrs |

## S10) Confirmation of trapped $^{14}CO_2$ using $BaCl_2$ test

In case the radioactivity detected in the NaOH trap exceeded 5 %AR a $BaCl_2$ test was performed in order to confirm mineralization. A volume of 1 ml of the NaOH solution was mixed with 50 ml of 0.05M $BaCl_2$, in a 50 mL tube and was shaken vigorously at 200 rpm for 30 min. During this process, the $^{14}CO_2$ absorbed in the NaOH solution would precipitate to $Ba^{14}CO_3$. After shaking, the solution was taken for centrifugation at 4000 rpm for 10 min and an aliquot of the suspension was taken for liquid scintillation counting.

## S11) Radio-HPLC method

The sample injection volume was 50 µL with the flow rate of the mobile phase set to 0.5 mL/min. For decane analysis an isocratic elution was performed with the total run time of 10.0 min and a solvent ratio of 10%A and 90%B (Solvent A: UHQ Water, Solvent B: Acetonitrile) was used. As decane is UV inactive, it was only detected in $^{14}C$ detector at a retention time of 5.13 min. For tetralin analysis a gradient elution (0.0-6.0 min: 95%A and 5%B, 6.00-18.0 min: 30%A and 70%B, 18.0-20.0 min: 95%A and 5%B) was used. The UV detector was set to measure the absorbance at three different wavelengths i.e. 195, 220, and 266 nm. The retention time recorded for the tetralin on the $^{14}C$ detector was 12.65 min and on the UV detector was 12.94 min.

## S12) Degradation time series of tetralin main study

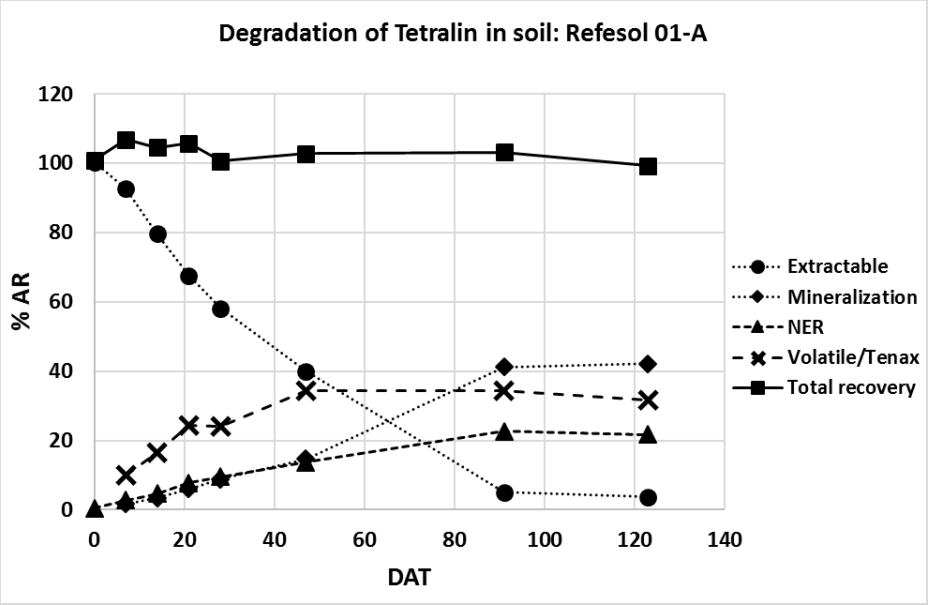



*Figure S4 Degradation of tetralin in soil: Distribution of radioactivity for soil 01-A in non-sterile samples*

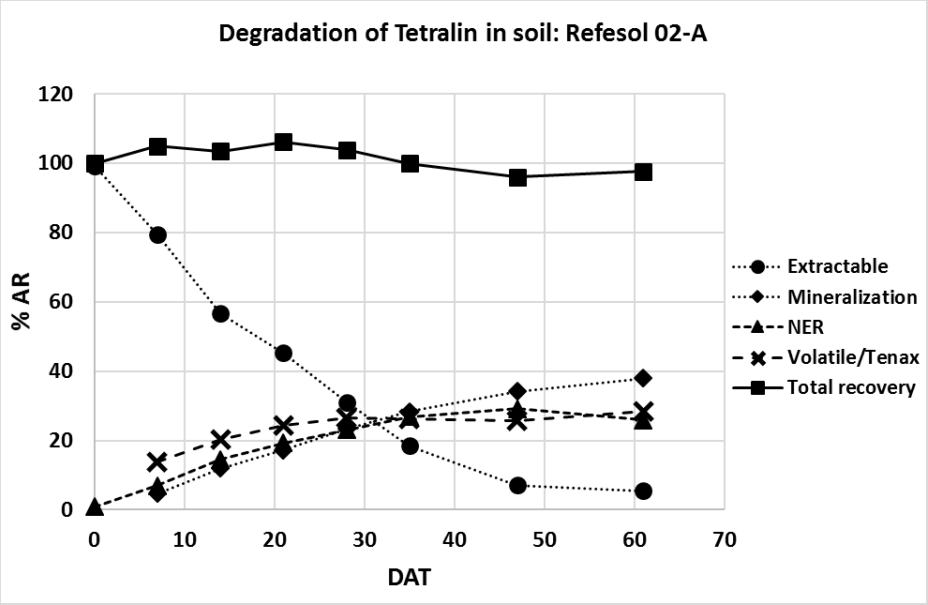



*Figure S5 Degradation of tetralin in soil: Distribution of radioactivity for soil 02-A in non-sterile samples*

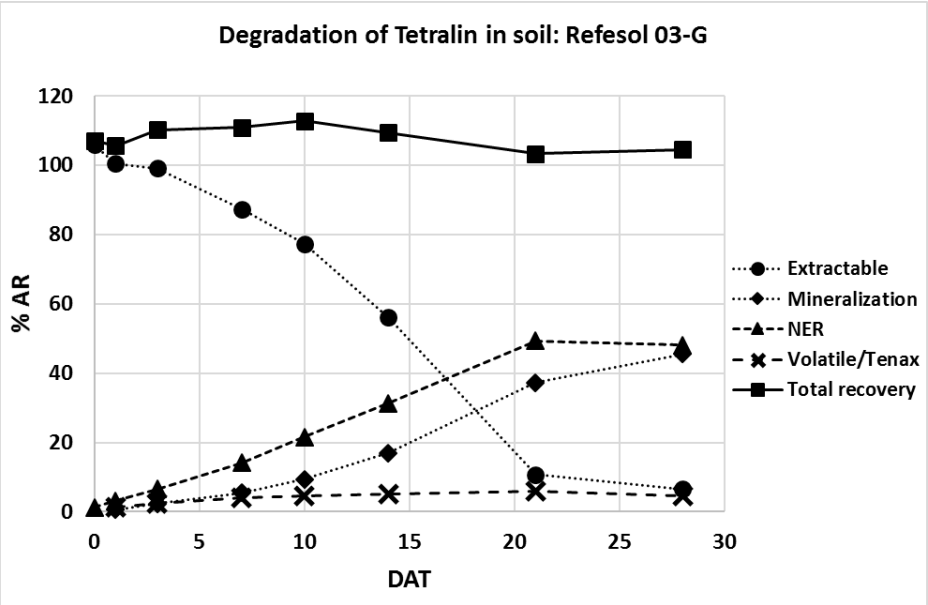



*Figure S6 Degradation of tetralin in soil: Distribution of radioactivity for soil 03-G in non-sterile samples*

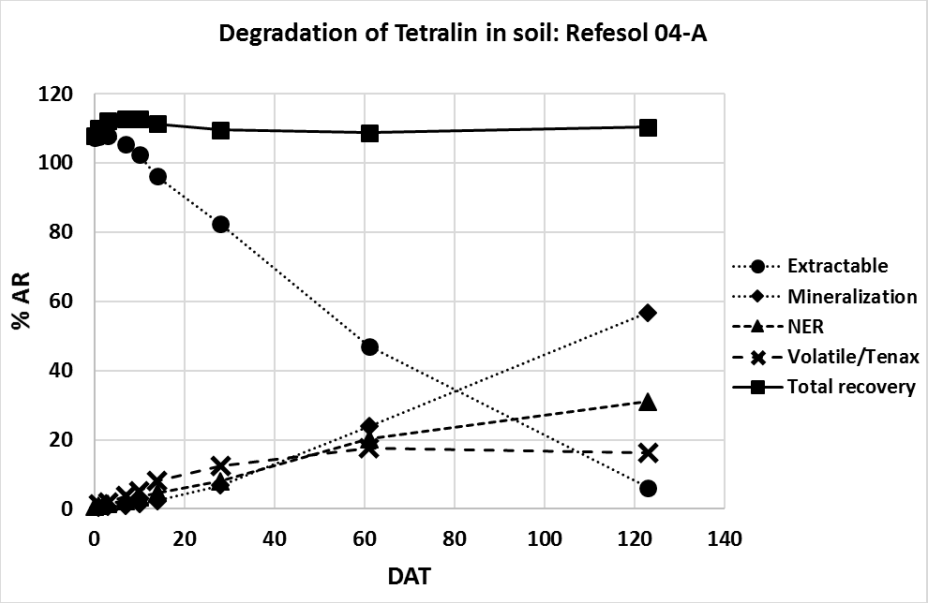



*Figure S7 Degradation of tetralin in soil: Distribution of radioactivity for soil 04-A in non-sterile samples*

## S13) Degradation time series of decane main study

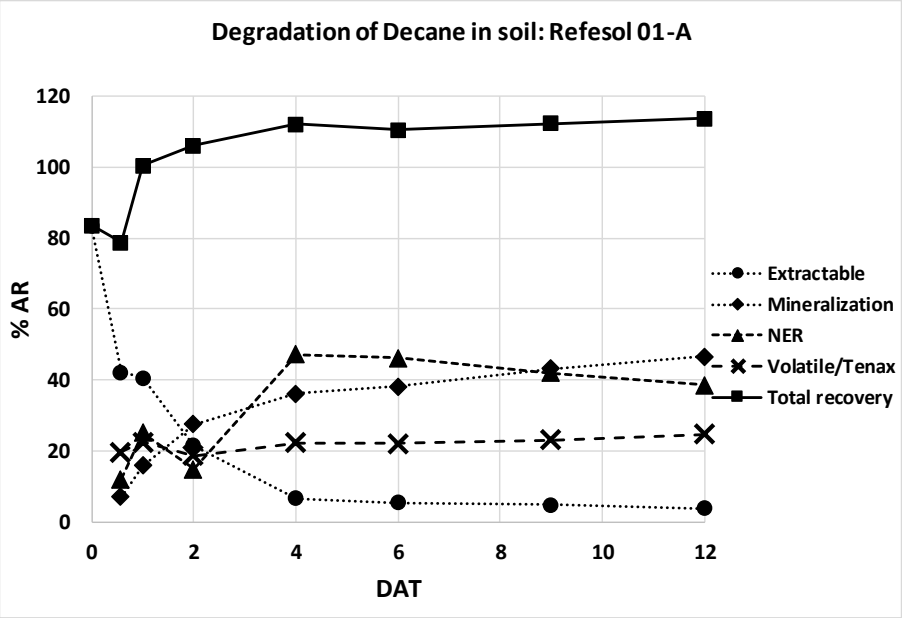



*Figure S8 Degradation of decane in soil: Distribution of radioactivity for soil 01-A in non-sterile samples*

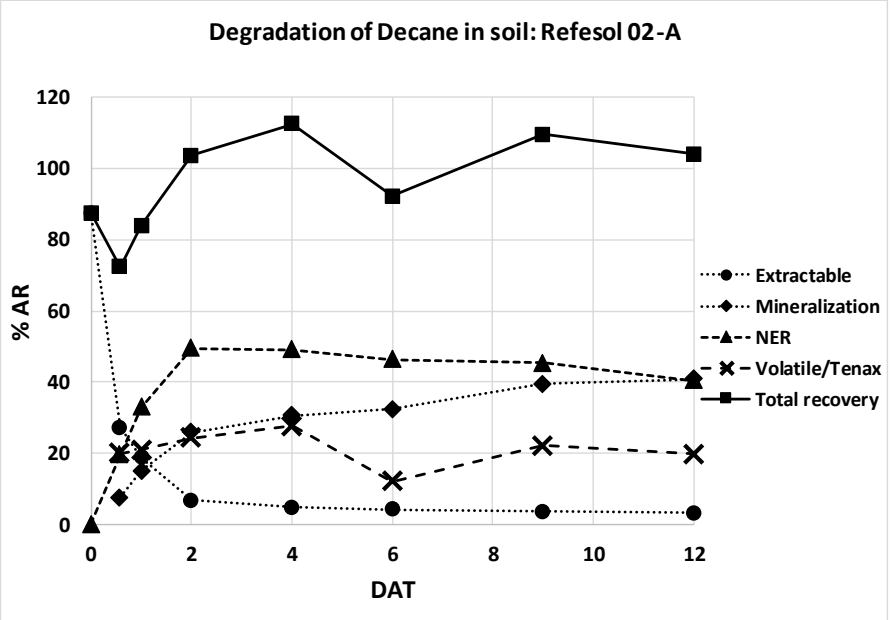



*Figure S9 Degradation of decane in soil: Distribution of radioactivity for soil 02-A in non-sterile samples*

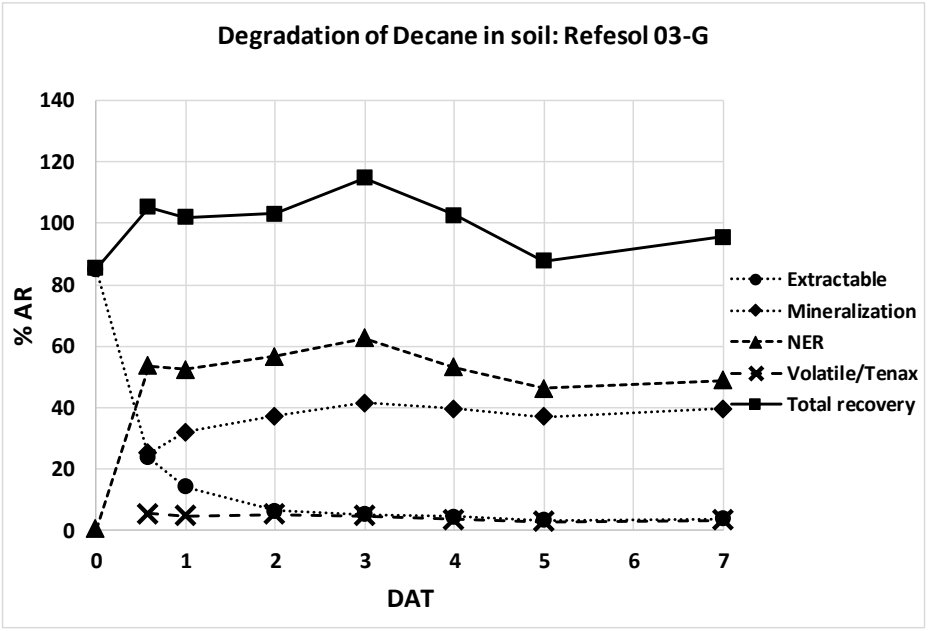



*Figure S10 Degradation of decane in soil: Distribution of radioactivity for soil 03-G in non-sterile samples*

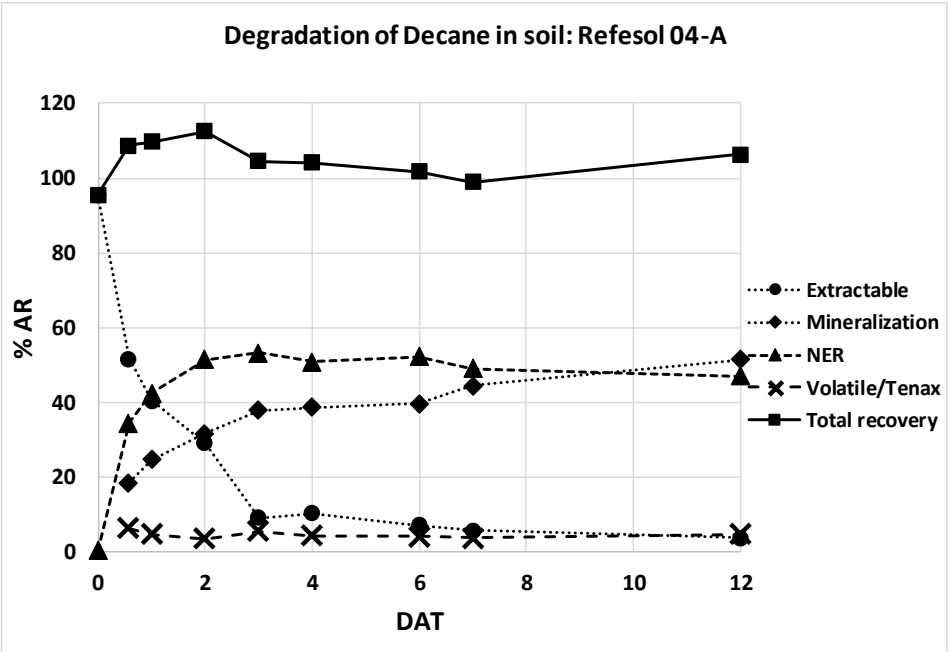



*Figure S11 Degradation of decane in soil: Distribution of radioactivity for soil 04-A in non-sterile samples*

## S14) Variability of results in terms of mass balance

*Table S3 Comparison of $^{14}C$-mass balances and variability of results in tetralin and decane main study*

| **Recovery** | **N** | **Decane** | **Tetralin** |
|---|---|---|---|
| **Recovery of all replicates [%aR]** | 90 [1)] | | |
| Mean | | 99.91 | 104.78 |
| Standard deviation | | 10.60 | 5.50 |
| Coefficient of variation [%] [2)] | | 10.61 | 5.25 |
| Amount of recoveries within 100 ± 15 %aR | | 88.88% | 100% |
| Coefficient of variation [%] between individual replicates – range | 90 | 0.01%-12.05% | 0.07%-9.1% |

*1) N = Total number of samples*
*2) Calculation: Coefficient of variation [%] = Standard deviation [%aR] of all replicates / Mean recovery [%aR] of all replicates × 100*

## S15) Comparison of radioactivity recovered in tenax in different soils in sterile samples

*Table S4 Comparison of volatilization of tetralin and decane in different soils in sterile samples*

| | **O.C content** | **Decane (days)** | **Tetralin (days)** |
|---|---|---|---|
| **01A** | 0.8 | 79.93 (14d) | 30.96 (28d) |
| **02A** | 0.98 | 87.13 (14d) | 40.84 (28d) |
| **03G** | 3.05 | 55.36 (14d) | 15.13 (28d) |
| **04A** | 2.79 | 34.08 (14d) | 3.93 (14d) |

## S16) Degradation kinetics

### Standard modelling

The scheme of the standard model is presented in Figure **Error! Reference source not found.**S12. First, kinetic analyses were performed using all available kinetic models, namely single first order (SFO), first order multi compartment (FOMC), hockey stick (HS), and double first order in parallel (DFOP). The calculation of DegT50 and DegT90 values were based on the fraction of radioactivity in extracts which could be identified as tetralin by radio-HPLC analysis. The results of the kinetic calculations for all four soils are summarized in Table **Error! Reference source not found.**S5.

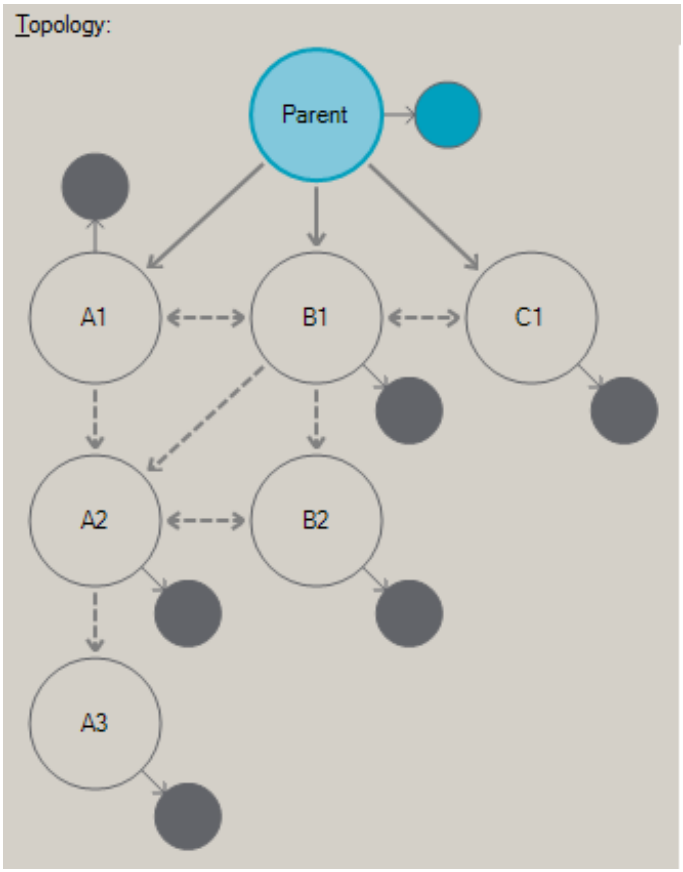



*Figure S12: Structure of the model used for optimization*

*Table S5 CAKE results for calculation of degradation rates $DegT_{50}$ and $DegT_{90}$ of tetralin in four different soils*

| Kinetic model | $DegT_{50}$ [days] | $DegT_{90}$ [days] | $\chi^2$ | Prob >t | |
|---|---|---|---|---|---|
| | | | | 1) | 2) |
| Soil refesol 01-A | | | | | |
| **SFO** | **15.3** | **50.6** | **4.47** | **2.44E-013** | |
| DFOP | 15.3 | 50.6 | 5.15 | 0.4999 | 0.5 |
| HS | 15.0 | 52.4 | 5.04 | 2.77E-009 | 1.33E-004 |
| FOMC | 13.6 | 45.0 | 4.76 | N/A | N/A |
| Soil refesol 02-A | | | | | |
| **SFO** | **9.4** | **31.2** | **15.0** | **9.21E-010** | |
| DFOP | 9.4 | 31.2 | 17.3 | 6.57E-009 | N/A |
| HS | 9.6 | 31.9 | 16.6 | 2.12E-008 | 0.4999 |
| FOMC | 7.9 | 26.4 | 16.0 | N/A | N/A |
| Soil refesol 03-G | | | | | |
| **SFO** | **7.3** | **24.4** | **7.14** | **3.02E-007** | |
| DFOP | 7.3 | 24.4 | 9.0 | 3.33E-006 | N/A |
| HS | 8.9 | 16.8 | 2.77 | 3.70E-006 | 4.81E-004 |
| FOMC | 6.4 | 21.3 | 7.87 | N/A | N/A |
| Soil refesol 04-A | | | | | |
| **SFO** | **28.6** | **94.9** | **5.0** | **1.71E-009** | |
| DFOP | 28.2 | 104 | 5.74 | 0.3573 | 0.5 |
| HS | 28.6 | 85.2 | 4.42 | 0.3528 | 2.77E-008 |
| FOMC | 28.5 | 96.6 | 5.34 | N/A | N/A |

1) for $\alpha$ [1] (FOMC kinetic model); for k1 (hs and DFOP kinetic model)
2) for $\beta$ [2] (FOMC kinetic model) ; for k2 (hs and DFOP kinetic model)

Based on the results it could be concluded that the SFO kinetics (marked in bold) shows generally the best performance as indicated by minimum $\chi^2$ – errors.

In addition to the standard model with consideration of the parent fraction in extracts only, an extended model was applied taking into account also the fraction of parent adsorbed on the tenax.

[1] $\alpha$ = Shape parameter determined by coefficient of variation of k values

[2] $\beta$ = Location parameter

**Extended modelling**

In addition to parent residues the sum of extractable metabolites and the volatile parent fraction were available and was considered further using an extended model, which is presented in the next figure (A1 = metabolites , B1 = volatilized parent residues). The analyses for the metabolites were based on the best-fit kinetics for the parent compound (SFO).

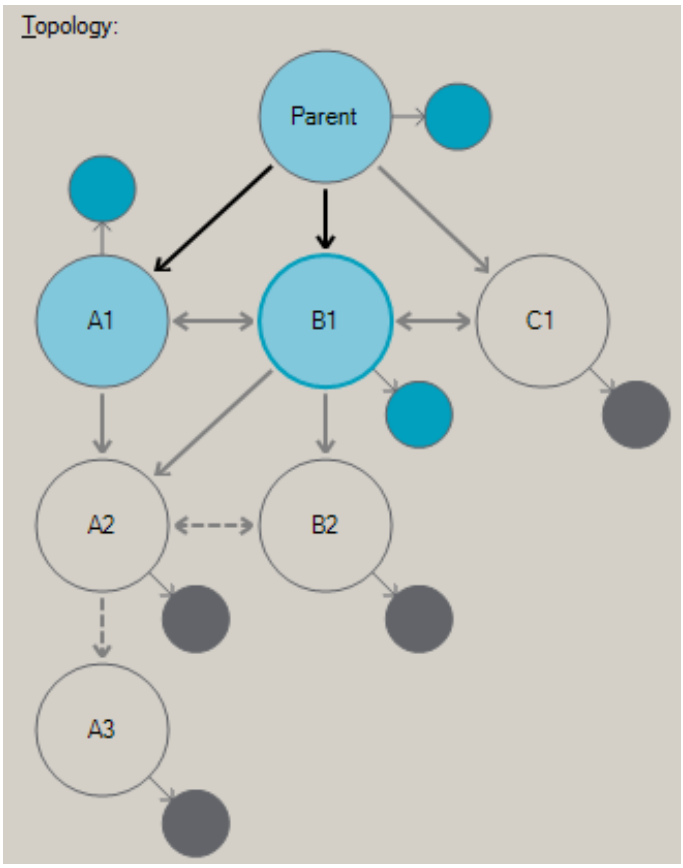



*Figure S13: Structure of the model used for the extended model (A1 = metabolites, B1 = volatilized parent residues)*

**S17) Microbial biomass measurements results during the main tests**

Results for the microbial biomass at the different stages are listed in Table S6 for tetralin and Table S7 for decane study. Microbial biomass determinations at study start (0d) were conducted with control samples untreated with solvent. At further stages of the study additional samples treated with the same amount of organic solvent as the samples applied with test chemical were analyzed in parallel to assess possible effects of the solvent to the microbial community in the respective soil. Biomass results indicated viable microflora at study initiation with $C_{mic}/C_{org}$ ratios > 1 % for all soils with exception of the 04-A soil in tetralin study, which was slightly below the threshold set by the test guideline. The measurements during and at end of the study demonstrate that the test system remained viable throughout the study, with exception of the 04-A soil, which was clearly below the required threshold.

*Table S6: Microbial biomass determined by means of substrate induced respiration (SIR) at different stages of the test during tetralin main study*

| Soil | Soil sample | Start of test (0d) | | During test* | | End of test** | |
|---|---|---|---|---|---|---|---|
| | | mg $C_{mic}$/kg dry mass | $C_{mic}/C_{org}$ (%) | mg $C_{mic}$/kg dry mass | $C_{mic}/C_{org}$ (%) | mg $C_{mic}$/kg dry mass | $C_{mic}/C_{org}$ (%) |
| 01-A | untreated | 83.5 | 1.1 | 81.5 | 1.1 | 46.7 | 0.6 |
| | treated with solvent | NS | NS | 161.1 | 2.2 | 237.4 | 3.3 |
| 02-A | Untreated | 214.0 | 2.1 | 165.6 | 1.6 | 194.6 | 1.9 |
| | Treated with solvent | NS | NS | 645.6 | 6.4 | 1254.2 | 12.4 |
| 03-G | Untreated | 434.6 | 1.6 | 417.0 | 1.5 | 428.7 | 1.5 |
| | Treated with solvent | NS | NS | 794.0 | 2.8 | 543.3 | 1.9 |
| 04-A | Untreated | 153.3 | 0.6 | 232.9 | 0.9 | 76.9 | 0.3 |
| | Treated with solvent | NS | NS | 135.0 | 0.5 | 177.2 | 0.7 |

NS: not sampled

* for soil 01-A: 28d; for soil 02-A: 28d; for soils 03-G and 04-A: 14d

** for soils 01-A/04-A: 123d; for soil 02-A: 61d; for soil 03-G: 28d

*Table S7: Microbial biomass determined by means of substrate induced respiration (SIR) at different stages of the test during decane main study*

| Soil | Soil sample | start of test (0d) | | During test* | | End of test** | |
|---|---|---|---|---|---|---|---|
| | | mg $C_{mic}$/kg dry mass | $C_{mic}/C_{org}$ (%) | mg $C_{mic}$/kg dry mass | $C_{mic}/C_{org}$ (%) | mg $C_{mic}$/kg dry mass | $C_{mic}/C_{org}$ (%) |
| 01-A | Untreated | 119.0 | 1.5 | 86.3 | 1.1 | 165.5 | 2.1 |
| | treated with solvent | NS | NS | 165.5 | 2.1 | 134.8 | 1.7 |
| 02-A | Untreated | 205.1 | 1.9 | 209.6 | 1.9 | 488.2 | 4.5 |
| | Treated with solvent | NS | NS | 247.2 | 4.0 | 186.0 | 1.7 |
| 03-G | Untreated | 523.2 | 1.8 | 473.2 | 1.6 | 872.1 | 3.0 |
| | Treated with solvent | NS | NS | 881.5 | 3.1 | 531.3 | 1.8 |
| 04-A | Untreated | 236.3 | 0.8 | 207.2 | 0.7 | 488.2 | 1.6 |
| | Treated with solvent | NS | NS | 370.5 | 7.2 | 330.8 | 1.1 |

NS = not sampled
* = 7 d
** = 14 d

**S18) Prediction of bio-NER using MTB and comparison with total NER from the tests**

An excel based program for prediction of bio-NER using MTB method was obtained from (Trapp et al. 2018) The required fields and entered values for the calculations have been listed below in the table.

*Table S8 The data entries made for the prediction of bio-NER using MTB method for tetralin and decane*

| **Test Chemical** | **Required fields :** Entered data |
|---|---|
| **Tetralin** | **Name**: Tetralin<br>**Structure**: C10H12<br>**molar mass g/mol**: 132.2<br>**Delta G0 of ATP**:-80 kJ/mol ATP<br>**Y ATP**: 5 g dry cell per mol ATP<br>**DeltaG0-values**<br>**Substrate S1**: 630.3 (n=1) kJ/mol<br>**Number of C atoms**: 10<br>**Number of H atoms**: 12<br>**Number of CH bonds**: 12<br>**measured $CO_2$%** : (see Table S9 below) |
| **Decane** | **Name**: Decane<br>**Structure**: C10H22<br>**molar mass g/mol**: 142.28<br>**Delta G0 of ATP**:-80 kJ/mol ATP<br>**Y ATP**: 5 g dry cell per mol ATP<br>**DeltaG0-values**<br>**Substrate S1**:924.9 (n=1) kJ/mol<br>**Number of C atoms**: 10<br>**Number of H atoms**: 22<br>**Number of CH bonds**: 22<br>**measured $CO_2$%** : (see Table S9 below) |

*Table S9 Total NER versus bio-NER predicted using MTB method for decane and tetralin*

| **Test Chemical** | **Parameter (% AR)** | **01-A** | **02-A** | **03-G** | **04-A** |
|---|---|---|---|---|---|
| **Tetralin** | **% Mineralization** | 8.79 | 23.33 | 45.48 | 6.83 |
| | **% total NER** | 9.53 | 23.04 | 48.05 | 8.0 |
| | **% bio-NER** | 6.70 | 17.76 | 34.72 | 5.21 |
| | **(bio-NER : total NER) Factor** | **0.70** | **0.77** | **0.72** | **0.65** |
| **Decane** | **% Mineralization** | 46.60 | 40.90 | 39.60 | 51.40 |
| | **% total NER** | 38.60 | 40.40 | 48.80 | 47.00 |
| | **% bio-NER** | 68.20 | 59.80 | 58.00 | 75.30 |
| | **(bio-NER : total NER) Factor** | **1.77** | **1.48** | **1.19** | **1.60** |