Methods Draft

# Bacterial Isolates

8 sets of bacterial isolates (Table A) were selected, sourced principally from the Nash’s Field experimental site at Silwood Park. Plots N and P (see Appendix X) of Nash’s Field have since 1991 been treated thrice yearly with metaldehyde at 960 g.ha-1 as part of herbivore exclusion experiments (Allan and Crawley, 2011), while bacteria were isolated and sequenced in 2016 for an earlier study (Mombrikotb, 2016).

|  |  |  |  |
| --- | --- | --- | --- |
| Strain | Species | Source | Exposure History |
| SBM\_R2A\_LUF4\_5 | *Luteibacter rhizovicinus* | Nash’s Field | Control |
| R2A\_KUB5\_13 | *Variovorax paradoxus* | Nash’s Field | Control |
| R2A\_NUF1\_3 | *Variovorax paradoxus* | Nash’s Field | Metaldehyde |
| R2A\_KUE4\_4 | *Bacillus muralis* | Nash’s Field | Control |
| R2A\_NUE1\_1 | *Bacillus muralis* | Nash’s Field | Metaldehyde |
| R2A\_KUE4\_10 | *Stenotrophomonas acidaminiphila* | Nash’s Field | Control |
| E. coli OP50 | *Escherichia coli* | C. elegans feeder | Control |
| Nash’s Field Community | Mixed | Nash’s Field | Control |

*Table A: Bacterial species, strains, and sources.*

Isolates were cultured in a standard R2A broth prior to experiments.

# Stressor Exposures

Stressor combinations were formulated by use of a Hamilton MicroLab STARLet. 255 combinations of 8 stressors across 8 bacterial isolates were formulated across 24 2uL 96-well plates. 10 μL doses of stressor solutions at environmentally relevant concentrations (Table B) were added to the wells, in addition to 10 μL of overnight bacterial culture diluted to 1 in 1000, and sufficient R2A broth to bring all well volumes up to 100 μL. Machine-readable worklists (Appendix Y) were generated from a combination input file for the STARLet using an R script (Appendix Z).

|  |  |  |  |
| --- | --- | --- | --- |
| Stressor | Environmental Concentration | Source | Form |
| Chloramphenicol | 50.0 ng/L | Choi *et al.*, 2008 | Powder, 98% |
| Amoxicillin | 120 ng/L | Andreozzi *et al.*, 2004 |  |
| Atrazine | 250 ng/L | Loos *et al.*, 2010 | Powder, analytical |
| Metaldehyde | 500 ng/L | Kay and Grayson, 2014 | Powder, analytical? |
| Copper | 20 mg/kg | Ross *et al.*, 2006 | Cu(II)Cl2, dihydrate (99%) |
| Nickel | 20 mg/kg | Ross *et al.*, 2006 | Ni(II)Cl2, anhydrous (98%) |
| Benzo[a]pyrene | 200 μg/kg | Ross *et al.*, 2006 | Solution in acetone |
| Benzene | 200 μg/kg | Ross *et al.*, 2006 | Liquid, 99.8% |

*Table B: Stressors, environmental concentrations, states and qualities.*

# Cell Counts

Immediately after isolates were exposed to stressors, plates were placed in a stacker-fed [NAME] optical density reader for 48h, agitating the wells for 5 seconds then reading absorbance at 600nm every hour. Optical density readings were converted into cell counts through the use of calibration curves generated by prior analysis performed on a [NAME] flow cytometer.

# Statistical Analysis

Optical density and flow cytometry results were processed to produce a measure of maximum slope over the course of the experiment, using a growth curve interpretation script from Mombrikotb (2016).

Maximum slope was graphed against number of stressors to produce initial graphs, while variation was modelled using linear models with different levels of interaction (2-way, 3-way, etc.) in the standard R package (R Core Team, 2017). Models were compared using ANOVA to determine how well they explained observed variation.