# 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). Isolates were cultured overnight on a rotary shaker in a standard R2A broth at room temperature prior to experiments.

|  |  |  |  |
| --- | --- | --- | --- |
| 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 | Multiple | Nash’s Field | Control |

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

# Stressor Rangefinding

Initial dose-response exposures were conducted at concentrations above and below environmental levels to obtain an overview of individual stressors’ effects on isolates. Eight 96-well microtiter plates were prepared, with each well being aliquoted with 10 μl of 1-in-100 diluted overnight culture, 80 μl of R2A broth, and 10 μl of stressor stock at either 10-1, 1, 101 or 102 times the target final experimental concentration. Each well of stressor concentration and isolate was replicated three times. Well OD was read using the cell count protocol below (3). Data is available in Appendix X.

# Cell Counts

Microcosm optical density was used as a metric of cell count over time. Immediately after isolates were exposed to stressors, plates were placed in an automatically-fed BioTek Synergy 2 microplate reader for 48 hours, agitating the wells for 5 seconds then reading absorbance at 590 nm every hour. Optical density readings were converted into cell counts through the use of calibration curves generated by prior analysis performed on a BD Accuri C6 flow cytometer.

# Stressor Exposures

Stressor combinations were formulated by use of a Hamilton MicroLab STARLet, fitted with sterile conductive pipette tips. 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.*

# 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.